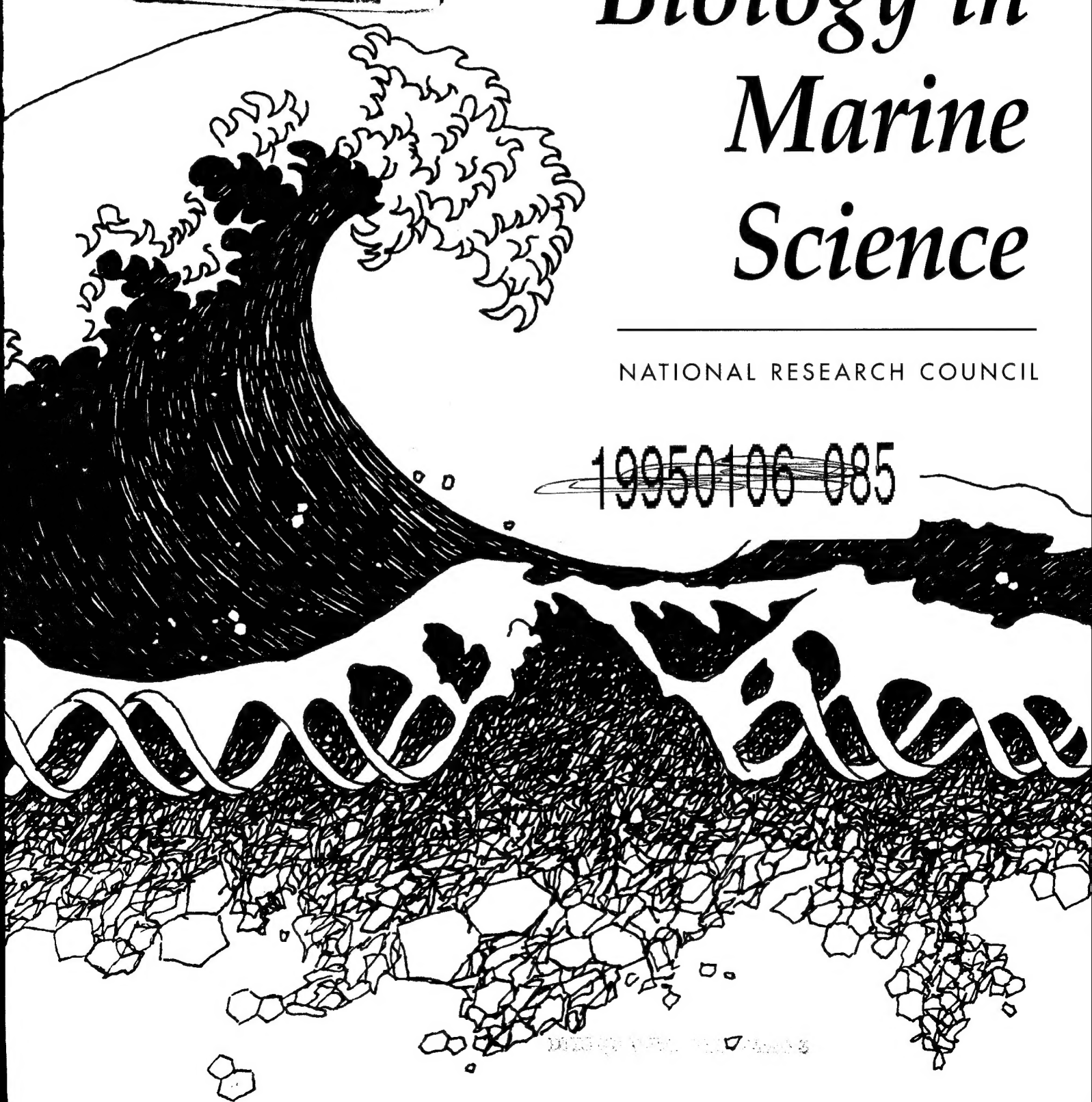


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Molecular Biology in Marine Science

NATIONAL RESEARCH COUNCIL

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MOLECULAR BIOLOGY IN MARINE SCIENCE:
SCIENTIFIC QUESTIONS, TECHNOLOGICAL APPROACHES, AND
PRACTICAL IMPLICATIONS

Committee on Molecular Marine Biology
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PREFACE

This report describes molecular techniques that could be invaluable in addressing process-oriented problems in the ocean sciences that have perplexed oceanographers for decades, such as understanding the basis for biogeochemical processes, recruitment processes, upper-ocean dynamics, biological impacts of global warming, and ecological impacts of human activities. The coupling of highly sophisticated methods, such as satellite remote sensing, which permits synoptic monitoring of chemical, physical, and biological parameters over large areas, with the power of modern molecular tools for "ground truthing" at small scales could allow scientists to address questions about marine organisms and the ocean in which they live that could not be answered previously. Clearly, the marine sciences are on the threshold of an exciting new frontier of scientific discovery and economic opportunity.

The ocean covers over 70 percent of the earth's surface and constitutes 90 to 95 percent of the biosphere by volume. It is a tremendous reservoir of high-quality food, biomedically important substances, organisms capable of degrading industrial and urban wastes or providing renewable nonpolluting energy, antibiofouling and anticorrosion substances, biosensors, biocatalysts, biopolymers, and many other industrially important compounds and products. Exploitation of the economic potential of the ocean could open frontiers of economic opportunity for entire new industries focusing on pharmaceuticals, specialty chemicals, aquaculture, and environmental engineering. The fundamental knowledge gained from basic molecular research on marine organisms will provide new opportunities for future developments important to the American economy, quality of life, and environmental health.

In February 1990 the National Research Council sponsored a workshop to evaluate the potential application of molecular biological "tools" to problems in marine biology and biological oceanography. Workshop participants, representing diverse disciplines, identified molecular techniques that could greatly enhance the effectiveness of marine research sponsored by the National Science Foundation, the Office of Naval Research, the National Oceanic and Atmospheric Administration, the U.S. Department of Energy, and other agencies by providing more incisive tools than were presently available. Workshop participants felt that the use of these technologies would usher in an exciting era of oceanography and recommended that a National Research Council committee be formed to explore in more depth this important area.

As a result, the Committee on Molecular Marine Biology was established (1) to assist interested federal agencies in program planning and the development of research priorities in the area of molecular marine biology and (2) to review how molecular biological tools could be applied to advances in the marine sciences. Specifically, the committee proposed to produce a report detailing the basic research needs of the ocean science community, describing how molecular techniques could help answer fundamental questions about the biology of marine organisms and expand our understanding of oceanographic processes, and outlining the potential benefits to society that may result from such advances. The committee intended to strengthen the partnership between two vastly different scientific communities—oceanography (biological oceanography and marine biology) and molecular biology. A barrier to the transfer of molecular technologies from the molecular sciences into the ocean sciences was the fundamental philosophical, intellectual, and scale perception differences between biological oceanographers and molecular biologists. Biological oceanographers study oceanic plant and animal life in relation to marine environments that often range from small to global scales, while molecular biologists study biological events in terms of the physiochemical properties of molecules in a cell which tend to be measured at microscopic or submicroscopic scales. A third group of scientists, marine biologists, who study the basic biology of organisms that inhabit the sea, are a natural bridge between biological oceanographers and molecular biologists because they tend to use a broad array of technologies and scales that range from molecular to global in nature. Marine biology is a broad discipline that intersects the fields of biological oceanography, physiology, cell biology, biochemistry, and molecular biology. The committee, which was composed of representatives from the three scientific disciplines, believed that biological oceanographers and molecular biologists, as well as the scientific community at large, would greatly benefit by the establishment of a closer working relationship or partnership between these scientific disciplines. Partnerships between such vastly different parties are often difficult initially; however, the committee believed that the advantages of a linkage between molecular biologists and oceanographers, with its tremendous potential for the ocean sciences in terms of information and technology transfer, well outweigh the cost of the effort. Although there continue to be some difficulties with effective communication, these difficulties will be resolved with continued effort and dialogue.

This report is the result of committee discussion and debate stemming from the different philosophies of oceanographers, marine biologists, and molecular biologists. The report was laid out at the National Research Council workshop, reworked by the committee, and modified after external review. The distinction between biological oceanography and marine biology and the question of whether

the study of marine organisms as model systems is part of marine science were particularly difficult to resolve. The fact that marine organisms are used for basic studies outside "traditional" marine science does not mean that it is **not** marine science or should not be targeted in a molecular marine science initiative. To bridge such disciplinary gaps, a broader vision among all scientists is needed. The committee recognized that many researchers working on model marine systems are cellular-molecular biologists, not oceanographers, yet we tried to point out that the fundamental biology revealed by such studies is essential for an understanding of many oceanographic processes.

Because of the divergent perspectives of the individuals involved in producing this report and the extensive discussion necessary to reach a consensus document, this report has taken several years to complete. As a result, several of the techniques and approaches described as imminent have already begun to make their way into marine science laboratories. However, their exploitation remains limited. Society is also becoming more aware of the implications of advancing marine science with molecular tools. For example, the U.S. House of Representatives passed the Marine Biotechnology Investment Act in 1993, authorizing an expenditure of over \$20 million per year for the next several years on basic marine biotechnology research through the National Oceanic and Atmospheric Administration's Sea Grant Program. Although the Senate has not passed a similar bill, it is hoped that a compromise bill will become law in 1994.

If biological oceanographers, marine biologists, and molecular biologists can continue to work toward a strong partnership during the next decade and recognize the intellectual and technological advantages and benefits of such a partnership, the twenty-first century will usher in an exciting new frontier of scientific achievement and economic opportunity for America that will rival the computer and biotechnological revolutions of the past two decades.

Dennis Powers, *Chairman*
Committee on Molecular Marine Biology

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EXECUTIVE SUMMARY

The ocean plays an important role in regulating the earth's climate, sustains a large portion of the earth's biodiversity, is a tremendous reservoir of commercially important substances, and is used for a variety of often conflicting purposes. In recent decades marine scientists have discovered much about the ocean and its organisms, yet many important fundamental questions remain unanswered. Human populations have increased, particularly in coastal regions. As a result, the marine environment in these areas is increasingly disrupted by human activities, including pollution and the depletion of some ecologically and commercially important species. There is a sense of urgency about reducing human impacts on the ocean and a need to understand how altered ecosystems and the loss of marine species and biodiversity could affect society.

During the past two decades, the development of sophisticated technologies and instruments for biomedical research has resulted in significant advances in the biological sciences. While some of these technologies have been readily incorporated into the study of marine organisms as models for understanding basic biology, the value of molecular techniques for addressing problems in marine biology and biological oceanography has only recently begun to be appreciated. The exploitation of molecular biological techniques will allow difficult research questions about marine organisms and ocean processes to be addressed and will offer new capabilities to tackle problems associated with global climate change, biodiversity, environmental quality, and responsible use of living marine resources.

The knowledge gained from basic research on marine organisms could also provide a basis for new commercial opportunities of economic importance for future generations. Immediate benefits could include improved management of fisheries resources, enhanced production of useful species through aquaculture, isolation and development of useful natural products from marine organisms, better information for the mitigation of environmental degradation, and the development of predictive models that will permit responsible management of marine resources.

This report defines critical scientific questions in marine biology and biological oceanography, describes the molecular technologies that could be used to answer these questions, and discusses some of the implications and economic opportunities that might result from this research which could potentially improve the international competitive position of the United States in the rapidly growing area of marine biotechnology. The committee recommends that the federal government provide the infrastructure necessary to use the techniques of molecular biology in the marine sciences. In particular, the committee makes recommendations in four areas, summarized below and explained in greater detail in Chapter 6.

Recommendations

Research Needs

This report identifies a suite of critical scientific questions in the fields of marine biology and biological oceanography. The committee has targeted seven basic research topics that it believes could benefit from increased attention and more appropriate facilities for carrying out research based on molecular techniques.

- **Quantification of inter- and intraspecific genetic variations for assessing species biodiversity, population structure, migratory movements, and gene flow. Of particular importance are DNA-based data for commercially important fishes, other species that are indicators of ecosystem health, and critically endangered species.**
- **Clarification of the role of marine viruses in marine ecosystems in light of their potential importance in ocean processes.**
- **Determination of effects of the environment on physiology and adaptation, especially the mechanisms regulating gene expression at the molecular level.**
- **Elucidation of metabolic pathways in marine organisms that lead to the synthesis and degradation of secondary metabolites and contaminants.**
- **Investigation of the role of chemical signals in the marine environment, including their chemical nature, detection, and potential usefulness to humans.**

- Investigation into the basic biology of a series of "keystone" marine organisms in order to develop techniques for assessing their physiological status in relation to recruitment processes, biogeochemical cycles, and other oceanic processes.
- Investigation of how eutrophication, toxic discharges, global change, and other human-induced environmental disruptions affect the abundance, distribution, and ecological success of species (i.e., biological diversity).

Technology Development, Technology Transfer, and Infrastructure

Some advances in fundamental biological knowledge have been achieved through the development of new molecular technologies. The development of these technologies has occurred outside the marine sciences. Technologies to solve many of the complex problems faced by marine scientists, therefore, either do not exist or must be redesigned for marine science. Thus, more effective mechanisms for encouraging the transfer of molecular biological technologies into marine science laboratories must be developed, if this field is to fulfill its potential. There are three aspects of this process: development of new technologies, transfer of new technologies into the marine sciences, and provision of infrastructure (e.g., facilities, equipment, study organisms).

Technology Development: The technology development upon which marine science depends will occur in many places—commercial, government, and academic laboratories—with a wide range of foci, including biomedical research, agricultural research, and marine science research. Although the committee does not make recommendations regarding the mechanisms required to accomplish this development, it does make a number of recommendations identifying research areas that would benefit from technology development.

- Couple molecular methods with new detection systems and computer-controlled robotic systems, so that large numbers of samples can be analyzed rapidly.
- Determine the potential usefulness of marine viruses as vectors for the genetic manipulation of marine organisms.
- Choose a series of key model marine organisms for comprehensive molecular-level study of developmental processes throughout their life cycles.

Technology Transfer: Technology transfer implies the application to marine science of techniques developed originally for use in other areas. Components of technology transfer include education and training, as well as mechanisms to adapt molecular techniques developed outside marine science for the study of marine organisms and processes. The technologies and approaches of molecular biology could contribute to existing federally funded initiatives, such as the Joint Global Ocean Flux Study, the Ridge Interdisciplinary Global Experiment, the Land Margin Ecosystems Research program, and the Global Ocean Ecosystems Dynamics program. Specific recommendations call for:

- **Developing technology to enable manipulation of organisms on board ships and in the laboratory under in situ environmental conditions.**
- **Maintaining and strengthening research fellowships and traineeships in molecular marine biology. A training program for midcareer biological oceanographers and marine biologists who desire to use molecular biological techniques in their research should be established. The National Oceanic and Atmospheric Administration would benefit greatly by establishing a marine biotechnology graduate fellowship program through its Sea Grant Program, with an applied science focus to study environmental change, land-sea interactions, water quality and productivity, habitat quality and restoration, and health of living resources.**

Infrastructure: Appropriate infrastructure will be necessary to promote development of new technologies and to provide opportunities for their use in marine science. Infrastructure modernization will be necessary for many marine research and teaching facilities in the United States. It is recognized that responsibility for these tasks belongs in some cases to the federal government and in others to universities, scientific societies, industry, and individual scientists. There is an opportunity for collaborative efforts among government, academia, and industry in molecular marine biology research and infrastructure development. The committee makes three specific infrastructure-related recommendations:

- **Increase the availability of equipment and instrumentation needed to enable marine ecologists and biological oceanographers to perform molecular studies.**
- **Improve the basic infrastructure of undergraduate and graduate teaching laboratories, to include modern instrumentation, and provide new facilities and laboratories where necessary.**

- **Modernize U.S. facilities for the culture of marine organisms to ensure a supply of critical microorganisms, marine algae, plankton, and marine animals for the studies recommended in this report.**

Public and Commercial Applications

A mechanism is needed to promote collaborative partnerships among federal agencies, academic marine scientists, and private industry and to permit appropriate research findings on marine organisms to be transferred rapidly to the private sector for commercialization. Private sector participation in funding research efforts and infrastructure could speed the development of bioremediation and environmental monitoring methodologies and could promote basic research on the biochemistry of novel compounds and metabolites that might be useful for biomedical applications. The private sector could also benefit from participation in a partnership with marine scientists and federal agencies to help support studies of marine biodiversity, which has the potential for exciting discoveries of biomedically important and/or environmentally useful organisms and compounds. In particular, the committee has identified three areas where the application of molecular biological techniques may lead to improvements in public health and/or the development of new products:

- **Better methods and indicators of chemical and biological contamination for screening contaminated waters, sediments, and seafood should be developed. This will allow monitoring of the safety of the marine environment and its living resources.**
- **Bioremediation methods should be explored using marine organisms or their gene products.**
- **Rapid screening methods for identifying and isolating biomedically useful compounds from marine organisms should be developed.**

Coordination of Support

The National Science and Technology Council, which replaced the Federal Coordinating Council for Science, Engineering, and Technology (FCCSET), provides a mechanism to integrate the biotechnology funding of federal agencies. FCCSET had been developing a coordinated national effort in biotechnology, including marine aspects, documenting the opportunities that will accrue by fostering research in marine biotechnology and promoting interagency cooperation. Although the U.S. investment in marine biotechnology is significant (about \$44

million in FY1992), it is modest compared with the efforts of some of America's most competitive international trading partners. Sensing the tremendous economic opportunity for marine biotechnology in the future, Australia, Norway, France, Germany, Israel, Japan, China, Taiwan, Thailand, and other European and Asian countries are spending hundreds of millions of dollars on marine biotechnology research and development (Myers and Anderson, 1992; Yuan and Hsu, 1993; Zaborsky, 1993).

The Committee on Molecular Marine Biology believes that, in order to answer the scientific questions posed in this report, achieve the scientific potential afforded by the techniques of molecular biology, and enhance the development and international competitiveness of the United States in the area of marine biotechnology, several actions will be necessary:

- Federal agencies, private industry, and academic scientists should work more closely together.
- The federal government should make an immediate long-term commitment to support molecular marine biology and biotechnology research and development.
- Adequate facilities and committed researchers are essential for scientific advances; the federal government, private sector, and scientists should work together to ensure that these physical and human resources will be available.
- Oceanographic programs with biological components, and individual scientists working on questions amenable to molecular approaches, should be targeted for education and financial support.

INTRODUCTION

The ocean covers over 70 percent of the earth's surface, comprises 90 to 95 percent of the biosphere's volume, and supports approximately half of the global primary and secondary food production. Marine organisms can degrade industrial and urban wasters and are a tremendous reservoir of food, substances important for medical purposes, renewable energy, fouling and corrosion prevention, biopolymers, production of biosensors and catalysts, and many other industrial applications. The fundamental knowledge gained from basic molecular research on marine organisms could fuel exciting new commercial opportunities and provide economic potential for future generations. Immediate potential benefits include improved management of marine resources such as fisheries, enhanced production of economically important species through aquaculture, development of useful processes and compounds from marine organisms, and new information of value for mitigating of environmental pollution and for resource utilization.

Human populations are increasing rapidly, and coastal ecosystems are being dramatically disrupted by human activities, including pollution and the depletion of some commercially important finfish and shellfish species. There is a sense of urgency about reducing human impacts on the ocean and a need to understand how altered ecosystems and the loss of marine species and biodiversity could affect society.

During the past two decades, the development of new technologies and instruments for biomedical research has been aimed at expanding our understanding of physiology, genetics, reproduction, development, disease, and nutrition. As a result of this research, significant insights and fundamental understanding of biological systems have been achieved. While some of these

new technologies have been readily incorporated into studies of marine organisms as models for understanding basic biology, the value and utility of molecular techniques for marine biology, ecology, and biological oceanography are only beginning to be appreciated (reviewed by Powers et al., 1990; Falkowski and LaRoche, 1991; Powers, 1993).

Among the significant contributions to our understanding of the basic biology of marine organisms that have been achieved are insights into the molecular mechanisms that regulate growth, reproduction, and development; environmental adaptation; the nervous system; information transfer and storage; immunological responses, and the synthesis and degradation of a range of biomedically valuable metabolites. In addition to fundamental scientific advances that will emerge as biologists continue to study marine organisms, new economic opportunities may become apparent in the areas of biotechnology, mariculture, and medicine.

Techniques of molecular biology will also contribute to our fundamental understanding of marine organisms and oceanic processes. For example, molecular techniques can be invaluable in addressing process-oriented questions in the ocean sciences that have perplexed oceanographers for decades, including understanding the basis for biogeochemical processes, larval recruitment, upper-ocean dynamics, marine biological diversity, ecological impacts of human activities, and the biological consequences of global warming. The physiological, biochemical, and genetic processes of individual organisms are often the most sensitive to environmental stress, and these processes are integrated into population- and ecosystem-level changes.

This report defines critical scientific questions in marine biology and biological oceanography, describes the molecular technologies that could be used to answer these questions, and discusses some of the implications and economic opportunities that might result from this research which could potentially improve the competitive position of the United States in the rapidly growing area of marine biotechnology (Figure 1).

Chapter 2 addresses the fundamental ecology of marine organisms, including the temporal and spatial distributions of marine organisms, how these distributions vary with time, the relationships between distribution and function, and the mechanisms that drive these processes. Chapter 3 discusses organismal responses to environmental conditions and issues related to studies of the physiological, biochemical, and genetic status of marine organisms. Chapter 4 details the use of marine organisms as models for biomedical research, some of the major discoveries of fundamental biological processes that have resulted, and the

"Critical Scientific Questions"

Basic Research
Biological Oceanography
Marine Biology
Cell/Developmental
Biology
Chemical Ecology

"Technologies to Answer Questions"

Molecular Technologies and Instruments
Technology Development
Technology Transfer
Infrastructure

"Practical Implications"

Applications and Social Impacts
Marine Biotechnology
Global Change
Environmental Quality
Marine Resources Management
Conservation
Products from the Sea

FIGURE 1-1. Overview of report structure.

potential advances in our understanding that may result from yet undiscovered marine organisms that will serve as model systems. Chapter 5 discusses the expanding field of chemical ecology, the role of chemicals in complex biological interactions, the nature of the products involved, and how these chemicals are produced, disseminated, and detected. In Chapter 6 the committee identifies basic research topics that it believes could benefit significantly from increased research support, recommends more effective mechanisms to encourage technology development and transfer, and proposes the need for a mechanism to promote collaborative partnerships among federal agencies, academic marine scientists, and private industry.

LIFE IN THE OCEANS: TEMPORAL AND SPATIAL DISTRIBUTIONS

Scientific Questions

Marine organisms, in general, have not been studied as extensively as their terrestrial counterparts, although studies of marine organisms have made major contributions to our fundamental understanding of ecological factors, such as keystone species, predation, and competition. Several fundamental questions underlie much of the research in biological oceanography today: What are the spatial distributions of marine organisms? How do these distributions vary with time? What are the relationships between distribution and function (i.e., production, metabolism, material cycling, and flux)? What are the mechanisms that drive these processes, including biological interactions, physical and chemical influences, and physiological responses of organisms?

Marine biologists have led the study of ecological interactions for several decades, but molecular techniques have not been widely used in this field. Molecular methods can allow us to proceed beyond descriptions of population structures to discern the mechanisms that govern population regulation, migration, and distributions. Understanding the control of spatial and temporal distributions of marine organisms will become increasingly important as we attempt to determine how natural and human-caused environmental changes affect population sizes, extinction, and invasion of new species. Although the need for such information is more obvious for species on which we depend directly for food or other uses, other species perform vital, if sometimes obscure, roles in ecosystems.

For the majority of marine species the abundance, genetic diversity, and spatial limits of populations have not been determined or are poorly understood. Knowledge of species distributions and genetic structure are important for (1) management of commercially important fisheries, (2) conservation and protection of endangered and threatened species, (3) assessment of the impact of global change and pollution on natural populations and the ocean's impact on global processes, (4) creation of models that describe and predict global energy flow and biogeochemical cycles, and (5) determination of the mechanisms of extinction and speciation in ancient and modern oceans and the resulting marine biodiversity.

Remarkable advances in our understanding of the problems outlined above have been achieved in the past decade by exploiting molecular biological techniques and approaches. The genetic variability of some populations of fish, cetaceans, sea turtles, phytoplankton, zooplankton, and other marine organisms has been revealed (reviewed by Falkowski and LaRoche, 1991; Powers, 1993). These studies demonstrate the promise of molecular techniques for answering questions that were previously intractable. Studies examining the genetic variability of the stocks of several species of commercially harvested fish e.g., (salmon, tuna, pollack, blue marlin) have demonstrated that new mitochondrial and nuclear DNA technologies will permit a definition of population structure, identification of "stocks," and quantification of gene flow. Such information is essential for effective management of oceanic fish populations.

Some fisheries, especially in North America, are declining, while others cannot be accurately assessed because of a lack of scientific data (U.S. Department of Commerce, 1992). Strategies such as the recent move toward moratoria on salmon fishing in the Pacific and swordfish fishing in the Atlantic can be avoided only if multinational management efforts are successful. Such management depends on knowledge, for each fishery, of the source (i.e., spawning grounds) of each fish, population sizes and genetic diversity that could produce a sustainable harvest, and the nature and extent of gene flow among separate populations, which may be critical to their long-term stability.

The ability to characterize genetic features of key marine species will illuminate the biology of these organisms by providing insight about their life history patterns, breeding structure, and the transfer of genetic information among populations. This information can also be compared with information about the variability of species abundance, ecosystem structures, and environmental conditions, allowing scientists to determine correlations and causal relationships among variables and to improve the models and techniques used by fisheries managers. In recent years molecular genetic characterization of endangered and threatened species, including cetaceans and sea turtles, has allowed better definition of population structures, genetic diversity of populations, and variability

in migration routes (e.g., Baker et al., 1990; Bowen and Avise, 1990; Meylan et al., 1990; Amos et al., 1991, 1992).

Molecular techniques also offer great promise for the study of microbial populations. Thousands of different species of phytoplankton, bacteria, fungi, and viruses existing in marine ecosystems comprise complex microbial food webs. These organisms are highly diverse in terms of the ecological and biogeochemical roles they play in elemental cycling and exchange within and between the ocean and atmosphere. Most of these species have not yet been named because they are not distinguishable morphologically from one another and cannot be cultured for taxonomic, ecological, and physiological studies.

Using RNA and DNA sequences to identify microbial groups, a data base describing the microbial diversity at the genetic level has been developed (Fox et al., 1977, 1980; Woese, 1987). Figure 2 shows one of the first phylogenetic trees determined from rRNA sequence comparisons. The genetic data base is being expanded rapidly as new sequences are added. In combination with phylogenetic probing and DNA sequencing, detection of functional genes that are similar to genes of well-studied species can help characterize these largely undescribed microbial species with respect to their ecological and biogeochemical roles in the marine environment. Because some species are difficult to culture, it is often easier to describe the genetic structure of organisms than to describe the organisms themselves. For example, DNA sequences that are uniquely found in archaeobacteria, a primitive group of bacteria previously thought not to occur in the surface of the oceans in appreciable numbers, have been obtained from seawater samples (e.g., Giovannoni et al., 1990). Furthermore, this approach has revealed the diversity and uniqueness of nonculturable symbiotic bacteria (e.g., Haygood and Distel, 1993). Use of this approach to study the diversity of important marine taxa other than microbes is becoming common (e.g., Palumbi and Benzie, 1991; Bucklin et al., 1992; Finnerty and Block, 1992; Silberman and Walsh, 1992).

Understanding the population dynamics of marine invertebrates has been hindered by our inability to distinguish among larvae of different species. Larval recruitment is a key event in the life cycle of most marine invertebrates and in the population dynamics of organisms that feed on them. Molecular taxonomic techniques have the power to advance our understanding of the ecology of these species greatly. In addition to facilitating studies of population structure and dynamics in marine organisms, molecular techniques have great potential for assessing the physiological status of individual marine organisms. In bacteria, for example, the amount of ribosomal RNA in the cell is related to growth rate and can be easily assayed by using fluorescent molecular probes. Finally, for photosynthetic organisms, the amount of the enzyme RuBP carboxylase in a particular cell can be an indicator of photosynthetic potential.

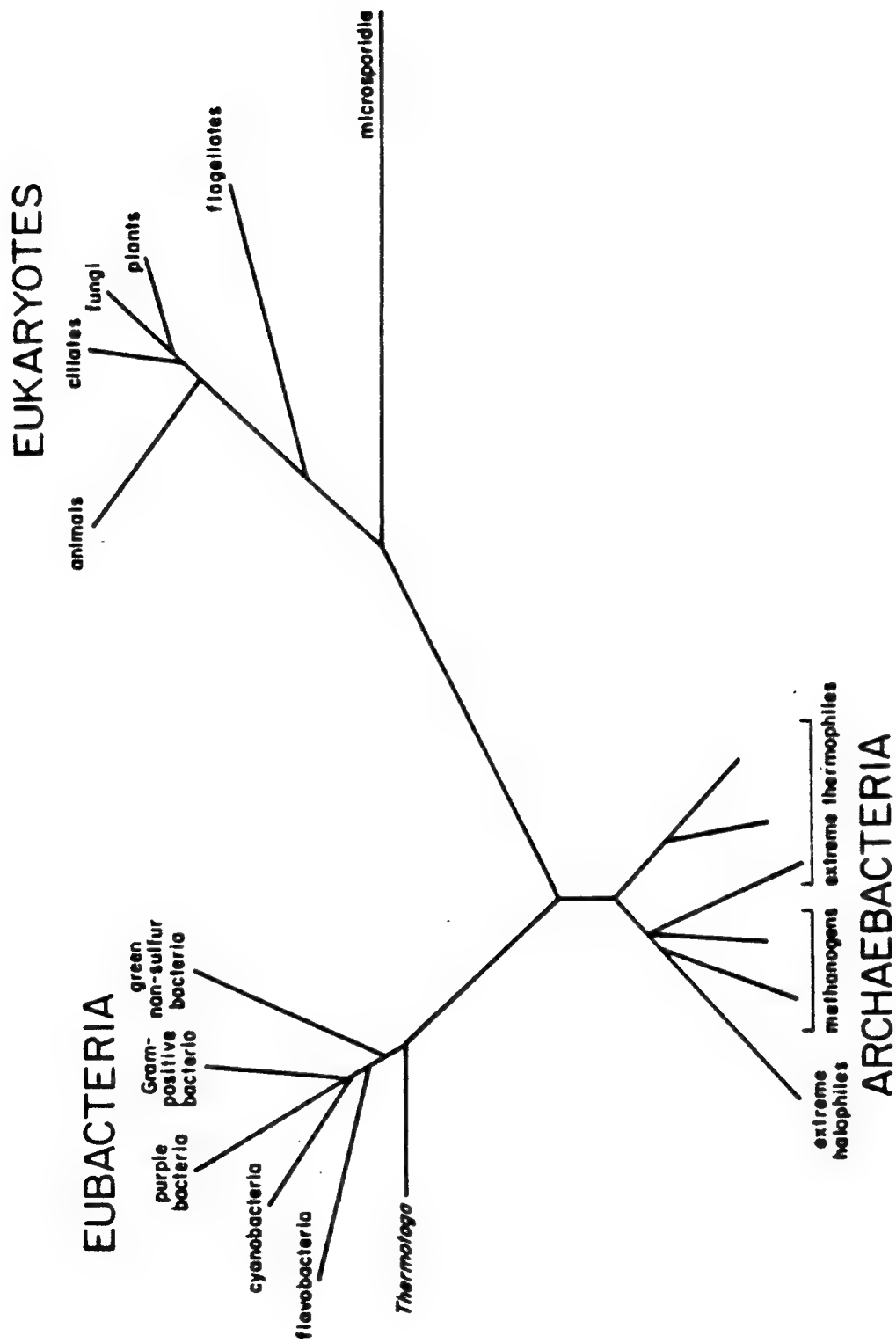


FIGURE 2-1. Universal phylogenetic tree determined from rRNA sequence comparisons (Woese, 1987).

Recent research has demonstrated that viruses are abundant in the ocean, being present at levels of between 10,000 and 10 million per milliliter of seawater (e.g., Proctor and Fuhrman, 1990, 1991). Little is known about their relationships with their host organisms, and thus the role they play in regulating the population dynamics of microorganisms in the sea is obscure. Because of the abundance of viruses, it has been suggested that viral infections may play a central role in marine bacteria and phytoplankton mortalities and in maintaining the diversity of microbial populations (Suttle et al., 1990). Our understanding of the biology of the ocean may be altered dramatically when the role of viruses is understood, in much the same way that our understanding of the human "ecosystem" was greatly enhanced after the relationship between viral infection and disease was established. Marine viruses offer tremendous potential for biotechnology as shuttle vectors, providing a vehicle to move selected pieces of DNA into a target organism.

Molecular tools could be used to study a variety of ecological processes, such as competition, predation, parasitism, and coevolution of host and parasites and predators and prey. The mechanisms that marine organisms use to select habitats are also an important topic that is just beginning to be addressed.

Techniques to Address the Scientific Questions

Several molecular methods have been developed for the analysis of proteins, RNA, DNA, and metabolites that can be applied to study how individuals, populations, and communities are distributed and to begin to assess the physiological status of individual organisms. The methods and techniques include (1) isozyme analysis; (2) immunochemical methods; (3) nucleic acid hybridization techniques; (4) restriction fragment length polymorphism (RFLP) analysis of mitochondrial, chloroplast, and/or genomic DNA; (5) DNA amplification by PCR; (6) DNA fingerprinting; and (7) DNA sequence analysis.

1. **Isozyme Analysis:** For almost three decades, electrophoresis of cell extracts followed by histochemical staining to identify specific protein electromorphs, has uncovered a wealth of genetic variations at the molecular level, both within and among species (Figure 3). These time-proven methods have continued to be useful to distinguish among species, analyze transport processes in the ocean, determine the genetic architecture of natural populations, study hybridization among species, and study the adaptive significance of isozyme variation in natural populations (e.g., Bucklin et al., 1989; Butth, 1990).

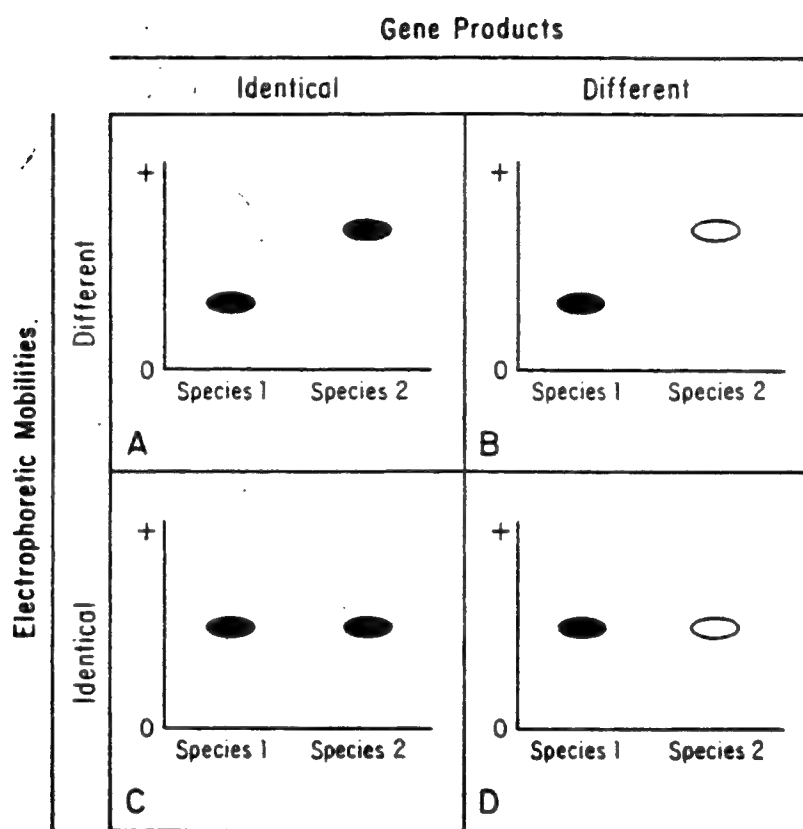


FIGURE 2-2. Theoretical combinations of electrophoretic mobilities and gene product identities (Buth, 1990).

2. **Immunochemical Methods:** Immunochemical methods can detect species- or population-specific molecules (antigens) on the surfaces of cells or in cell extracts and have been used to identify bacteria, phytoplankton, zooplankton, eggs, larvae, and adult marine organisms. One application of this technique has been for identification of aquatic nitrifying bacteria (Ward and Perry, 1980) and nitrogen-fixing microorganisms (Currin et al., 1990) and mapping of their distribution over large areas. In addition to the identification of species and life history stage, immunochemical methods have been used to describe community structure and predator-prey interactions, to identify the partially digested remains of prey organisms from within the digestive systems of their predators (Theilacker et al., 1986; Grisley and Boyle, 1988; Feller et al., 1990), and to detect parasites (Ohman et al., 1991).
3. **Nucleic Acid Hybridization:** Nucleic acid hybridization, like isozyme analysis, can be used to discriminate among organisms that lack distinguishing morphological features, such as microorganisms and larval forms of complex multicellular organisms. DNA hybridization can be designed to discriminate among major groups of organisms or to detect individual species within a group of closely related organisms (Woese, 1987). Probes that target unique DNA sequences are prepared and reacted with DNA from the organisms of interest. This technique has already been used to identify species in marine microbial populations (DeLong et al., 1989; Sanghoon and Fuhrman, 1990).
4. **RFLP Analysis:** RFLP analysis is used to characterize the genetic composition of organisms. Digestion of genomic DNA, mitochondrial DNA, or chloroplast DNA by enzymes that target specific DNA sequences (restriction enzymes) generates DNA fragments that can be separated by electrophoresis to form distinctive patterns that can then be analyzed by standard statistical techniques. Comparison of the similarity among patterns has been used to identify the geographic origin of individuals and to study the taxonomic relationships among species. Information gained by RFLP analysis is especially useful in fisheries management, conservation of endangered species, transport studies of plankton, and studies of evolutionary relationships. RFLP analyses have also been used to study the genetic diversity of a variety of marine and freshwater organisms, including bacteria, marine macrophytes, phytoplankton, zooplankton, eels, oysters, sea turtles, and fish.
5. **Polymerase Chain Reaction (PCR):** This technique has provided a revolutionary approach for the synthesis, detection, and characterization of specific sequences of DNA. The PCR procedure employs a heat-stable

bacterial DNA polymerase and specially constructed oligonucleotide primers to replicate specific DNA sequences in vitro. From as little as a single molecule of a target DNA sequence, enough material for standard analytical procedures such as RFLP analysis, gene mapping, DNA hybridization, DNA fingerprinting, or even DNA sequencing can be produced in about three hours. Advantages that can be gained by the use of PCR include (1) rapid detection and identification of microorganisms that occur at very low frequency (e.g., one or a few cells in a liter of water), symbiotic microorganisms, and minute individual fish and invertebrate larvae; (2) rapid analysis of individual genomes for population studies; (3) detection and analysis of "rare events" (e.g., gene rearrangements) that occur in a small fraction of cells in a tissue sample or field collection; and (4) estimation of water quality by detection of pathogenic viruses, bacteria, and/or parasites. Another advantage of PCR is that it eliminates the need to culture what are often found to be nonculturable microbial species and strains of ecological or biogeochemical importance. PCR currently is being used for biosystematics, population biology, conservation biology, ecology, developmental biology, and genetics (see reviews by Arnheim et al., 1990; Powers et al., 1990; Powers, 1993).

6. DNA Fingerprinting: Repetitive regions of DNA, called minisatellites, are dispersed throughout the genomes of a number of organisms. Jeffreys et al. (1985) showed that a subset of human minisatellites shared a common 10 to 15 base pair core that had hypervariable regions. Later they demonstrated that a nucleic acid hybridization probe could detect highly polymorphic minisatellites that could be used as DNA fingerprints specific to an individual. DNA fingerprinting is now commonplace in biomedical research and is routinely employed in a variety of legal situations. Moreover, the method is being used to address many scientific questions about terrestrial and aquatic organisms (reviewed by Ryland and Tyler, 1989; Burke et al., 1991; Powers, 1993).

Whales and other cetaceans have been studied more frequently than other marine species (e.g., Hoelzel et al., 1991; Schloetterer et al., 1991). However, DNA fingerprinting has also been applied to freshwater and marine fishes, invertebrates, and aquatic plants (reviewed by Ryland and Tyler, 1989; Powers, 1993). For example, Whitmore et al. (1990) used DNA fingerprinting to study sibling largemouth bass (*M. salmoides*). They showed that DNA hybridization patterns were different for each individual but that siblings were more similar to each other than to fish from wild stocks. Wirgin et al. (1991) developed striped bass-specific DNA probes, 10 to 20 base pairs in length, which they used to study bass population structure. One of the probes allowed them to distinguish between Gulf of

Mexico and Atlantic coast populations as well as discriminate among several of the Atlantic stocks. Turner and his colleagues (1991) have used repetitive DNA sequences to study the population structure of several fish species.

Several laboratories are using a PCR-based approach to amplify DNA sequence length polymorphisms that have DNA repeats of specific nucleotide combinations. Such approaches can be used to examine hundreds of individuals rapidly, at a modest cost. Moreover, the approach can be used for fresh, frozen, or alcohol-fixed samples from a broad array of geographic areas and from any particular life history stage (eggs, larvae, fry, or adult). Data generated by DNA fingerprinting techniques can be used for assessment of genetic stocks, for restoration and mitigation activities, and for the management of critical estuarine and marine resources.

7. **DNA Sequence Analysis:** DNA sequence analysis, in which the actual sequence of the DNA subunits (nucleotides) is determined, is used to study genetic relationships within and among marine communities, species, and populations. When PCR amplification is used to provide the DNA for analysis, the sequence information can be obtained without sacrificing the organism or, in the case of microorganisms, without culturing them in the laboratory. Nucleotide sequences of specific genes are also used to establish taxonomic relationships, to study the genetic architecture of natural populations, to manage endangered species, and to study the evolution of marine organisms. During the past five years, there has been an increase in these types of studies in the marine science community (reviewed by Avise, 1993; Powers, 1993).

Automation of Molecular Techniques

The efficiency and usefulness of molecular techniques are increased in those instances in which automation expedites the processing of large numbers of biological samples. Automation is important for processing samples for molecular analysis because it is often necessary to collect a large number of samples from a given study area. Large sample numbers when processes that vary greatly over time or space are being studied and/or when repeated sampling at the same site is required. A number of methods for protein and nucleic acid analysis have already been automated, and others are in the developmental stages as a result of the Human Genome Project and their commercial applications for diagnostic purposes in the biomedical field.

For many types of biological analyses, samples must be collected and preserved in the field and analyzed later in the laboratory. Automated techniques to replace human identification and enumeration of microbes, invertebrate larvae, and other organisms not readily identified on the basis of gross morphology could make near-real-time analysis of biological samples at sea or in the field a reality. A few of these automated methods are highlighted below.

Protein Isolation: Automation of methods for protein isolation is the paramount problem in characterizing proteins from complex living organisms, organelles, or even cells, requiring the separation of thousands of different proteins. The technique best suited for this type of analysis is two-dimensional (2D) gel electrophoresis, whereby tens of thousands of proteins may be separated in one dimension by size and in the second dimension by electrical charge. Computerized image analysis is presently used to digitize information from 2D gels so that it may be stored in the computer. This process allows comparison of different 2D gels to determine the qualitative or quantitative differences in the locations of protein spots. With this technique it will be possible to carry out, on a routine basis, 2D gel electrophoresis and subtractive analyses of marine organisms or cells. This type of analysis will be even more useful when fully automated 2D gel techniques are developed.

Once appropriate proteins have been identified for characterization, a technique termed "electroblotting" can be used to transfer proteins from 2D gels directly onto appropriate materials from which their amino acid sequences may be determined.

Protein Sequencing: Automated methods have been developed for sequencing proteins, allowing the determination of protein sequences at the subpicomole level. The current state-of-the-art sequencing technique, using a conventional gas-phase protein microsequencing instrument, permits the analysis of approximately 10 picomoles of material in a typical sample. New solid-phase sequencing instruments and techniques are currently being developed to permit the use of fluorescent phenylisothiocyanate (PTH) derivatives, enhancing the detection of PTH amino acid derivatives 10,000-fold and speeding the throughput of protein sequencing five-fold. Within the next few years, fluorescent sequencing and solid-phase techniques will permit analysis of proteins at the level of 100 femtomoles.

A second approach to protein microsequencing uses the mass spectrometer as a tool to isolate peptide fragments for subsequent sequencing. Techniques such as electrospray and plasmid desorption will permit, in the not-too-distant future, analysis of intermediate-size peptides (up to 40 residues) at levels that will probably fall well below the picomole level. The power of the mass spectrometer as a tool for analyzing peptides is that it can characterize peptides rapidly (in

minutes) and identify, in a general way, any secondary modifications that have occurred on proteins. After proteins are sequenced, standard techniques can be used to identify the genes corresponding to these protein products.

Both solid-phase and mass spectrometer approaches allow rapid processing, with cycle times that are as little as 15 to 25 percent of the present sequencing times. Thus, these combined techniques will allow many proteins from marine organisms to be characterized and their corresponding genes to be isolated.

DNA Mapping and Sequencing: Automated DNA mapping and sequencing methods have been developed that make DNA sequencing rapid, reliable, and accessible to a broader range of scientists. Fluorescent DNA sequencing technology allows analysis of between 40,000 and 60,000 bases per week by a single technician operating one machine. This output represents an enormous increase in the capacity for processing DNA sequences, so that genes from a large number of samples can be characterized on a greatly increased scale suitable for population studies.

New techniques for both genetic mapping and physical mapping are being developed to increase throughput and increase their potential for automation. For example, techniques are being developed whereby four different fluorescent dyes can be applied to DNA physical mapping. Because four different emission wavelengths can be used, three unknowns and one standard can be run in each lane of a pulsed field electrophoresis gel. Another technique, the oligonucleotide ligase assay, permits individual base polymorphisms to be analyzed. This technique has been automated for DNA mapping work with a robotic workstation.

Computational Tools: The advancement of techniques for analyzing protein and DNA sequences will require appropriate computational tools for the acquisition, storage, retrieval, and analysis of the resulting information. For example, specialized computer chips have been developed recently that have the capacity to detect patterns in DNA or protein sequence data at speeds that are orders of magnitude faster than possible with conventional computers. In addition, these chips can be attached to personal computers or to relatively simple workstations, as well as to larger computers. Other computational techniques, such as neural nets, can be developed to analyze informational patterns. Object-oriented data bases will permit the storage of many different types of data; differences in data type will be transparent to the user. One of the new computational problems in biology is the existence of many different data bases that must be transparent to provide easy access and movement through a variety of data bases to gather the desired information.

Molecular and/or pattern recognition systems (e.g., receptors, functional groups, specific antibodies, enzymes, and metal-binding proteins) can be associated with a monitor in such a manner that they generate an extensive array of molecular recognition elements, coupled via a series of signal transducers. Binding of specific molecules to these recognition elements causes a conformational change in the receptor molecule that is sensed and amplified by the transducer. Thus, the binding of specific molecules found in organism extracts to a specific recognition element can be detected. Such systems have the potential for accommodating a vast number of detector molecules or receptors. The potential of the system is presently limited by the dearth of fundamental biological information on specific molecules and their receptors in marine environments. As this information becomes available, coupling the recognition elements to an automated system could be easily accomplished and would permit rapid assessment of their presence in biological extracts or water samples.

Flow Cytometry: Flow cytometry, which has been used for cell analyses in biomedical research for the past 20 years (Shapiro, 1988), has been adapted for the study of marine organisms (Shumway et al., 1985; Chisholm et al., 1986, 1988; Olson et al., 1990). A new generation of multichannel flow cytometers is being developed with the capacity to identify and count an array of planktonic species automatically, as part of a shipboard multiinstrument water sampler. Submersible versions are in the design stages. Flow cytometers are being used at sea to separate and count selected species of plankton by either their natural fluorescence emission or their size or by detection of fixed cells with molecular probes possessing fluorescent reporters. This approach has expanded the ability to determine the spatial distributions of marine microbes on a scale previously impossible even with conventional microscopy. Applications of this analytical approach include water-quality monitoring, rapid and quantitative detection of indicator organisms, and monitoring of the distribution and abundance of selected microbial species in polluted estuaries and harbors by the enumeration of different metabolic groups of bacteria (e.g., sulfate reducers, denitrifiers, nitrogen fixers, nitrifiers, methanogens, or other microbes), many of which play critical roles in the biogeochemical cycles controlling greenhouse gases.

Microscopic Techniques: New microscopic techniques that afford high resolution can be exploited to determine functional relationships of organisms, to explore development, and to evaluate molecular mechanisms such as ligand-receptor binding. These microscopic techniques include (1) confocal, (2) atomic force, (3) optical force, and (4) scanning Fourier-transform infrared.

Practical Implications

The molecular techniques and approaches described above provide a means to address fundamental biological oceanography questions. Although some advances in our understanding of the distribution and function of marine organisms have been made, exploitation of molecular techniques could provide insight into critical issues facing marine science today, including global climate change, management and conservation of living marine resources, seafood quality, and bioremediation.

Global Change

One of the critical questions facing marine science today is the role of the ocean in global climate change. It is likely that the ocean and atmosphere behave as a coupled system and that changes in one induce changes in the other. Changes in climate could influence oceanic circulation patterns (Broecker, 1991), which could, in turn, change the patterns of productivity and dispersal of organisms in the ocean. Changes in the biological communities of the ocean could significantly alter biogeochemical cycles for elements such as carbon, altering atmospheric concentrations of carbon dioxide and other greenhouse gases. Without a relatively complete understanding of the factors that regulate the population dynamics of existing populations, we will have great difficulty predicting and/or monitoring changes in populations of marine organisms that might be affected by human activities.

Research programs have been initiated to define and describe the ocean's role in global change and the potential effect of global change on marine organisms; molecular biology can play a part in accomplishing the objectives of these programs. For example, a major goal of the Joint Global Ocean Flux Study is to gain an understanding of how carbon dioxide is exchanged between the atmosphere and the surface ocean and how carbon is cycled through physical, chemical, and biological effects and transferred to the deep ocean (Figure 4). Coastal and marginal seas, where greater than 50 percent of primary productivity occurs, are important carbon sinks (Mantoura et al., 1991), and research funded by the U.S. Department of Energy (DOE) and the National Oceanic and Atmospheric Administration could be enhanced by using molecular techniques for studying these processes. DOE has started a new initiative called Molecular Approaches to Ecosystems Research and has used this to augment its Ocean Margins Program, and the Office of Naval Research has a molecular biological component to its new Marine Environmental Quality program. Molecular techniques can be used to provide information about population size and community structure in surface waters and the deep ocean, which is essential to understanding carbon dioxide exchange and carbon cycling. In addition, they will

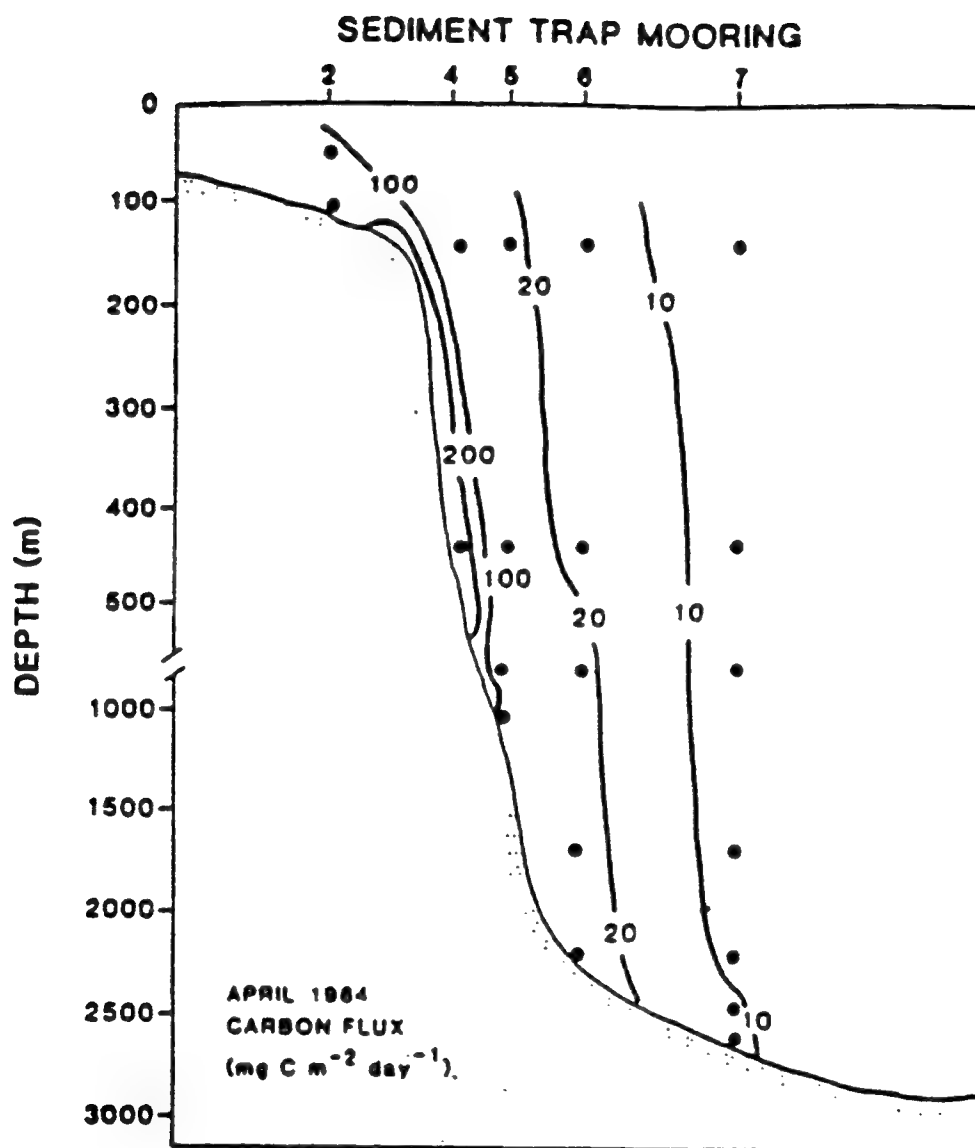


FIGURE 2-3.

The daily carbon flux caught in sediment traps moored on the continental shelf, slope, and rise of the Mid-Atlantic Bight during April 1984 (after P. Biscaye personal communication in U.S. JGOFS Long Range Plan, 1990).

be important in linking key primary producers with mineralizers controlling the net flux of carbon dioxide into coastal waters and its ultimate fate once fixed into complex organic molecules.

Resources

The ocean is a source of many resources, including food. As worldwide population grows, the demand for these resources will also increase. Many fish and shellfish populations throughout the world ocean are showing signs of decline due to both harvesting and disease (Figure 5). For example, standing stocks of some commercially important fish (tuna, swordfish, salmon, shark) that live in the ocean have declined sharply in recent years. Fisheries managers usually lack necessary data to demonstrate stock size, the genetic diversity of the population, and the amount of interbreeding among populations in different ocean basins. The degree to which fish populations are controlled by various environmental factors is poorly characterized, although there is some evidence that physical factors produce effects that ripple upward through marine food webs. For example, the Global Ecosystems Dynamics Experiment (GLOBEC) cites evidence that increased winds over the North Atlantic Ocean in the 1950s to 1970s decreased phytoplankton and zooplankton production, reducing the food supply and populations of some fish species (National Research Council, 1991). Information needed to answer questions of stock size and genetic diversity could be gathered by combining data obtained using molecular techniques with a better understanding of the physics and food web structures of ecosystems. Such information could also result in better fisheries management and possibly increased harvests of commercial fish stocks (Figure 6). A strong and productive fishing industry could increase the amount of fish and shellfish exported from the United States. The GLOBEC program will focus on how a changing global environment could alter the stability and productivity of marine ecosystems. GLOBEC planning has placed emphasis on the use of molecular techniques for its studies.

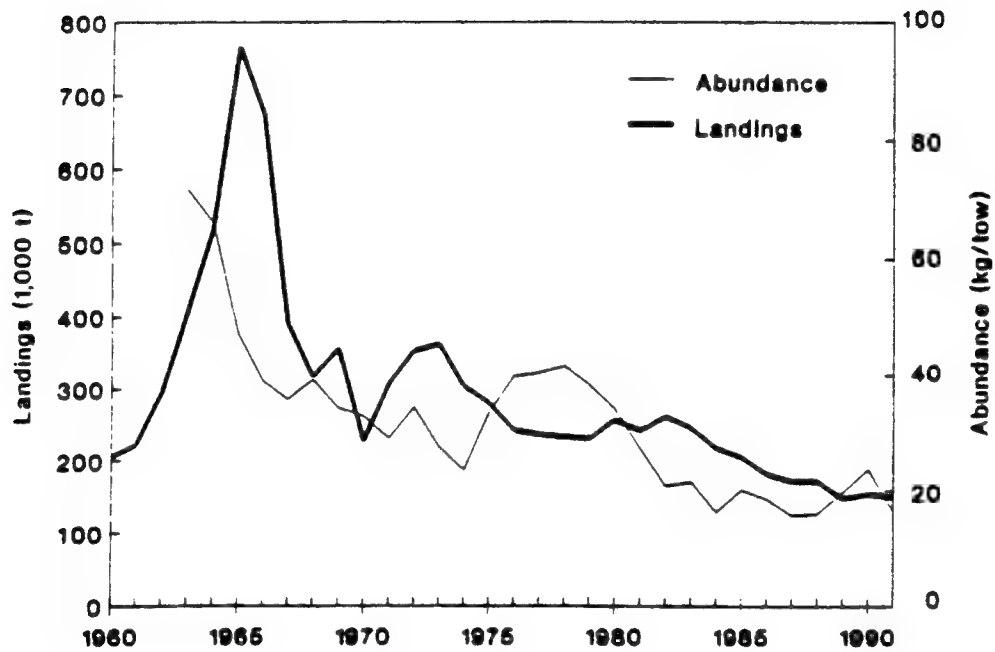


FIGURE 2-4. Total U.S. commercial landings and abundance indices for principal groundfish and flounders off the New England coast, 1960-1991 (U.S. Department of Commerce, 1992).

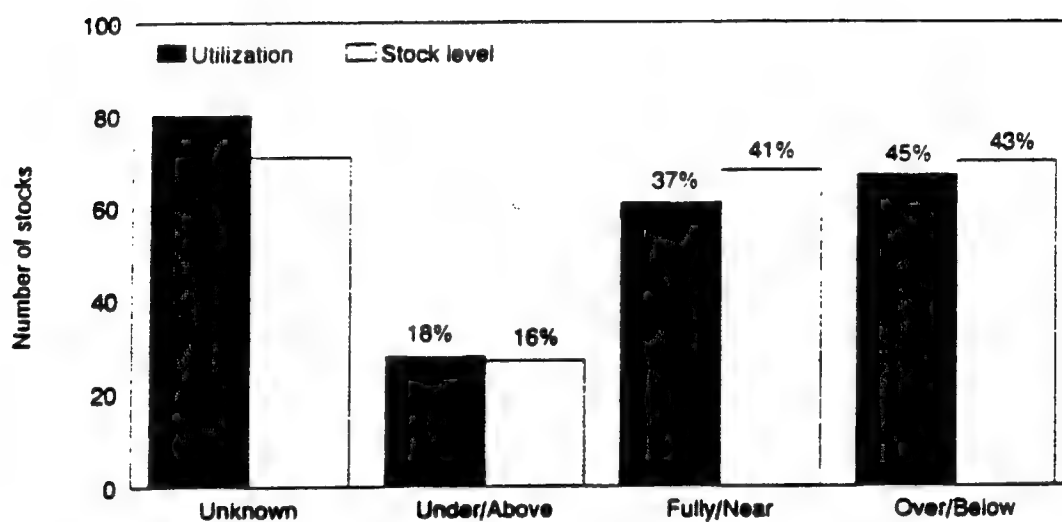


FIGURE 2-5. Status of U.S. living marine resources. Utilization and stock level relative to the level needed to support long-term potential yield. The bars represent the number of stocks, and the figures at the top of each bar are the percentage of stocks for which the status is known in that category of utilization or stock level (U.S. Department of Commerce, 1992).

PHYSIOLOGICAL, BIOCHEMICAL, AND GENETIC STATUS OF MARINE ORGANISMS

Scientific Questions

There are profound consequences of the strong relationships among the physical, chemical, and biological processes in the ocean. The ocean varies over time and space due to natural factors and human activities. The variability is evident in the latitudinal and vertical changes in the spectral intensity of light; in the gradients of temperature, pressure, and salinity; in the distribution of suspended particulate matter and of a multitude of chemicals such as biologically important nutrients, carbon dioxide, oxygen, and trace gases; in the distribution and abundance of organisms; and in the effects of ocean hydrodynamic factors such as currents, eddies, fronts, storms, and turbidity currents.

Some environmental variability is periodic and is the pacemaker for daily and seasonal events such as animal migrations, phytoplankton blooms, and sexual reproduction. Long-term change, detected by the study of sedimentary fossils, can be episodic, as in the case of the Permian-Triassic extinctions, or periodic, as in the sequence of major mass extinctions during the Phanerozoic. The triggering mechanisms for mass extinctions are not fully known, but primary productivity under the stress of global change may be an important factor. The response of marine organisms that inhabit the upper ocean to an increased flux of ultraviolet radiation due to depletion of stratospheric ozone is only now beginning to be studied (e.g., Cullen et al., 1992).

Marine organisms respond to environmental cues by behavioral, physiological, immunological, humoral, and genetic mechanisms. Ultimately, such responses at the level of individual organisms aggregate to produce

population-level effects, the focus of Chapter 2. *Study of responses by individual organisms to environmental conditions, both biotic and abiotic, is a fundamental aspect of marine biology and is necessary for understanding how both natural variability and human activities (e.g., global climate change and pollution) affect marine ecosystems.*

Knowledge about the effect of ocean processes on the cycling of biologically important elements, such as carbon and nitrogen, is important for estimating the potential impact of global climate change on marine systems. For example, the ocean is both a sink and a source in the global carbon cycle, with processes such as burial of organic carbon (Lyle, 1988) and dissolution of carbonates in marine sediments (Archer and Maier-Reimer, 1994) affecting atmospheric carbon dioxide on time scales of months to thousands of years. To understand the carbon cycle, we must understand the biochemical and molecular mechanisms that regulate metabolism and food webs.

Research on organismal responses to environmental conditions is focused on three questions: What is the physiological condition (e.g., nutritional, immunological, developmental, reproductive) of marine organisms? How do marine organisms respond to deviations in environmental factors (e.g., food, temperature, oxygen, dissolved nutrients, chemical contaminants, light, pressure, current velocity)? How do environmental factors regulate ocean processes (e.g., organic carbon burial, development of anoxic zones, nutrient regeneration, sea-air exchange of volatile compounds, primary productivity)?

Responses of organisms to environmental parameters (including other organisms) are believed to account for much of the variability in population dynamics and community structure in marine food webs. Organisms can respond to deviations in environmental conditions through changes in gene expression and metabolism that yield changes in physiological state and performance. These responses may result from changes in the expression of relevant genes. The responsiveness of gene expression to environmental factors is well documented for many terrestrial organisms but for only a few marine organisms (Davidson, 1986, 1989; Koban et al., 1991; Sanders et al., 1992). For most marine organisms, including those of major ecological and economic importance, the influence of environmental factors on biochemistry, physiology, and gene expression is virtually unknown. Understanding the molecular details of these regulatory processes could allow the development of genetic and immunological probes and biochemical tests to quantify the physiological condition and health of marine organisms and their responses to environmental stress. It is important to characterize sublethal effects (even though the subject organism is alive and appears healthy by other measures) because even small organismal effects can sum to large effects on the population

or global levels. This type of information is important for global biogeochemical models that include information about the biosphere and its interactions with the physical environment.

Patterns and mechanisms of gene expression may differ among taxa, so that studies of a broad range of marine organisms could demonstrate a variety of patterns of gene expression. For example, glycolytic pathways differ among marine invertebrates and between vertebrates and marine invertebrates.

Although today's molecular methods can identify genetic variation (Chapter 2), assessment of physiological status and the diversity of physiological processes of individual organisms within these communities is not easily accomplished. It is desirable to be capable of both identifying the species present and predicting the response of each species to a number of environmental variables. Development of appropriate diagnostic tests will require considerable fundamental knowledge about the basic biology of the target organisms, such as a description of their life histories and knowledge of sensitive life stages, determination of whether their reproduction and development depends on natural environmental cues, and identification of stress manifestations in the biochemistry and genes of these organisms. In the future, molecular techniques could help resolve questions related to environments that place unusual stresses on the physiology of marine organisms, such as high temperature, high salinity, and high pressure. Also of interest are how organisms change metabolically as they proceed through different environments in different life history stages--for example, salmon living as juveniles in fresh water, maturing in the ocean, and returning to fresh water to spawn.

Techniques to Address the Scientific Questions

Several molecular techniques can be used to study the physiological condition of marine organisms in relation to environmental parameters. Many of the key ecological and physiological attributes of natural populations, such as rates of reproduction, recruitment, growth, energy flow, and the level of environmental stress to which organisms are exposed, are difficult to assess directly, especially when large numbers of individuals must be analyzed. Because the physiology of organisms reflects underlying biochemical and molecular activities, indices of such activities should, in principle, provide useful means to assess physiological state and permit rapid analysis of large numbers of samples.

The development of biochemical and molecular indices of physiological state requires precise laboratory-based calibration, in which the environmental factor of

interest is varied and changes in biochemical and molecular properties are assessed quantitatively. When significant correlations are found between environmental variables and biochemical or molecular processes, useful indices can be developed. The use of nucleic acid concentrations and immunological techniques is described below; other methods (delineated in Chapter 2) are applicable for determining physiological status when applied to the appropriate biological function.

1. **Nucleic Acid Concentration:** Some ecological studies, including growth rate measurements, have relied on measurement of RNA and DNA concentrations and their ratios. Fisheries biologists have shown that under certain circumstances the ratio of RNA (over 95 percent of which is ribosomal RNA) to DNA can provide a rough estimate of growth rate (Bulow, 1987) and is a useful indicator of nutritional status (Clemmesen, 1990; Ueberschaer and Clemmesen, 1990). However, this technique is useful only if proper controls and standards are employed, including restricting comparisons to single species, size classes, life history stages, environmental temperature, and the physical activity of the individuals being tested.

Because the majority of the RNA pool is ribosomal RNA, ratios of RNA to DNA cannot be used to quantify the synthesis of specific proteins. Development of nucleic acid probes to quantify the synthesis of individual proteins could yield more specific indicators of physiological or biochemical status, such as growth rate, reproductive status, stress level, and nutritional state. Since the first step in protein synthesis is the production of messenger RNA (mRNA), mRNAs for any particular protein are represented by a tiny fraction of the total RNA pool. In order to quantify a specific mRNA species, it is necessary to clone and characterize a cDNA probe that will exclusively hybridize to the specific mRNA in question (e.g., Crawford and Powers, 1989). These cDNA probes can be used to quantify the relative concentration of a locus-specific mRNA (e.g., Crawford and Powers, 1989) and/or they can be used to determine transcriptional rates (e.g., Crawford and Powers, 1992) (Figure 7). As other locus-specific cDNAs are cloned and characterized, they can be used to determine the effects of short-term environmental changes on the physiological status of marine organisms. The major barriers to the expanded use of this methodology are (1) the paucity of fundamental knowledge about the protein-specific mRNAs that reflect physiological, biochemical, or genetic status, (2) attaining the levels of amplification (or signal detection) needed so that the signal can be quantified, and (3) the cloning and characterization of appropriate cDNA probes that can be used to quantify the specific mRNAs.

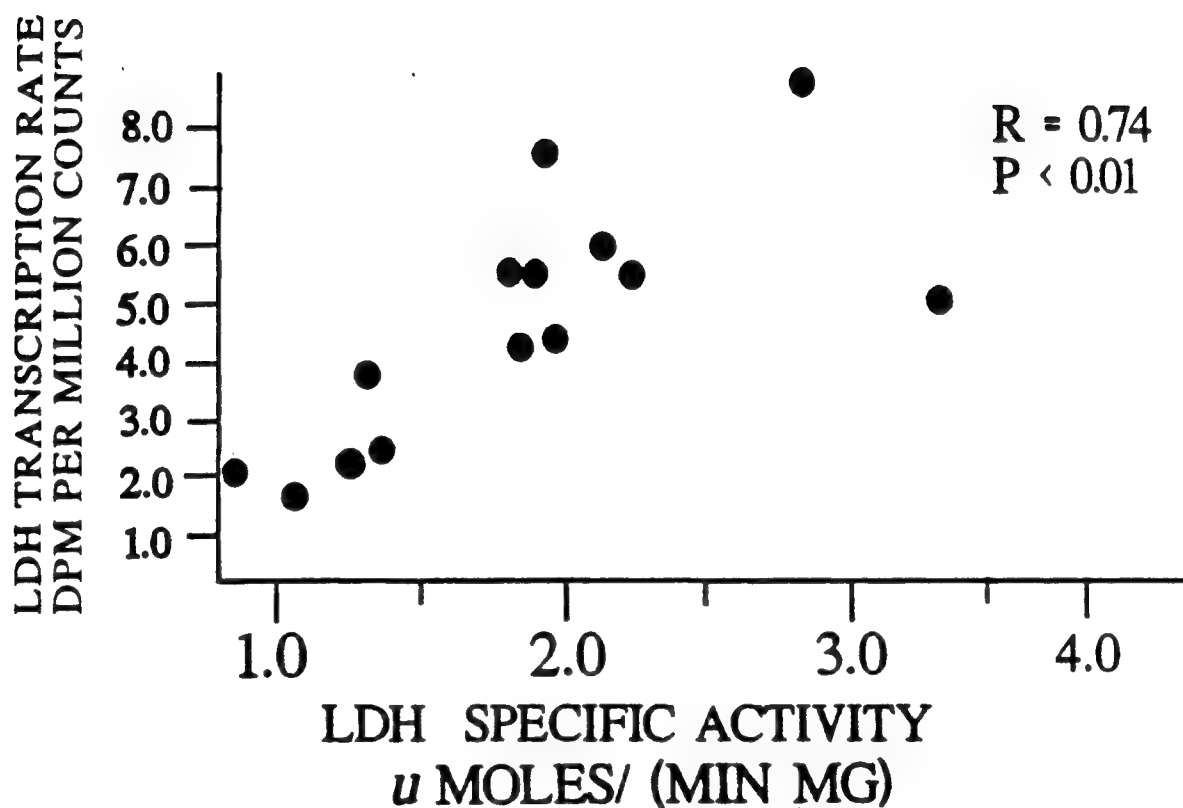


FIGURE 3-1. Relationship between transcription rate from the *Ldh-B* locus and the amount of LDH-B enzyme (Crawford and Powers, 1992).

2. **Immunological Techniques:** In addition to being useful for detecting species and population differences (Chapter 2), antibodies are effective tools for determining how environmental factors, such as temperature, salinity, organic toxins, and metals, affect the concentrations of specific proteins (heat shock proteins, cytochrome P450, metallothioneins, metabolic enzymes, growth and reproductive hormones, and others) (e.g., Chen, 1983; Gedamu et al., 1983; Koban et al., 1991; Sanders et al., 1992). Because some of these specific proteins occur with similar amino acid sequences in a range of organisms, antibodies can sometimes be designed to react and bind selectively with sequences common to several species. In other cases, it is necessary to develop species-specific antibodies. These immunological probes can then be used to monitor and quantify the impact of environmental variables on the physiological status of marine organisms. The main barriers to the use of immunological methods to assess the physiological, biochemical, or genetic status of marine organisms are (1) the identification of appropriate indicator proteins that reflect important physiological or biochemical processes and (2) the purification of these indicator proteins and the subsequent generation of antibodies directed against them.

Practical Implications

Variations and changes in the global environment from natural events and human-induced changes can produce effects on marine ecosystems with great biological and economic impacts. For example, El Niño events severely disrupt marine ecosystems and marine fisheries in the Pacific Ocean. Global changes could likewise affect marine organisms, yet specific physiological information required for predictive capabilities is lacking. Molecular techniques offer opportunities to identify environmental impacts on marine species by detecting and characterizing changes in the synthesis of important macromolecules such as proteins at transcriptional, translational, and product levels.

Physiological Condition of Marine Organisms

Knowledge of the physiological condition of organisms and how organisms respond to optimal and suboptimal environmental conditions could contribute to improved management of commercially important and endangered species, enhance monitoring for water quality and environmental change, and provide a means to monitor other species. Resource managers need information describing the physiological conditions of populations, how these conditions vary over time,

and how they are affected by the environment. These data, which can be obtained by using molecular techniques, will allow better marine resource management; potentially stronger, more productive industries; and responsible protection of marine resources.

Study of stress responses of marine organisms to environmental factors could lead to the development of methods to produce specialty chemicals (pharmaceuticals, enzymes, hormones), by "optimizing" stressful conditions. Many marine algae produce chemicals of commercial value in response to stressful external environmental conditions. For example, when certain species of marine algae are grown under salinity stress or nitrogen limitation, they produce large quantities of β -carotene, alcohols, glycerol, and hydrocarbons (e.g., Ben-Amotz and Avron, 1990; Bubrick, 1991; Behrens and Delente, 1991).

Mariculture

Even with improved fisheries management strategies, the catch of fish from the sea could decline. The culture of marine organisms (mariculture) could partially compensate for declines in wild populations (National Research Council, 1992). An understanding of how environmental stress affects the biochemistry and physiology of cultured organisms is essential for the successful expansion of mariculture. Such knowledge would allow mariculturists to monitor the health, nutritional state, and reproductive status of their crop to provide cultured organisms with an optimal environment. An understanding of the genetics and biochemistry of growth hormones and environmental impacts on hormone production and gene expression could enhance mariculture production and provide the foundational knowledge for eventual genetic engineering of organisms to increase the production of hormones and other biochemicals that increase growth or other desirable characteristics of cultured organisms (Fischetti, 1991).

Mariculture poses risks to adjacent environments, including release of waste products and excess food and the possible release of exotic (nonnative) species into coastal ecosystems. Molecular techniques can be exploited to reduce these problems. For example, molecular methods can be used to monitor waste effluents and then to treat mariculture waste in a manner similar to that used to treat urban waste. Molecular methods also can be used to monitor effluents from mariculture systems to prevent escape of exotic species to neighboring waters. Genetic engineering techniques can be used to ensure that cultured exotic species are reproductively sterile, so that they cannot reproduce if they inadvertently escape. Genetic manipulation of eggs can produce sterile triploid organisms that

also have the advantage in some organisms that growth is enhanced (Allen and Downing, 1986).

Environmental Quality

One of the consequences of the world's growing population is the degradation of highly productive coastal ocean ecosystems as they receive increasing discharges of plant nutrients such as nitrate and phosphate, metals and other toxicants, and particulate matter. Many ecologically and economically important species spend all or a portion of their lives in these threatened habitats. Bioremediation can be an effective means to restore the natural condition of coastal ecosystems. Bioremediation could benefit greatly from identification and characterization of relevant genes encoding key proteins and enzymes involved in biodegradation. An improved understanding of the physiology and biochemistry of marine organisms capable of transforming contaminants could enhance our ability to restore marine habitats. The impact of the introduction or stimulation of selected species in natural populations could also be predicted, in part, if the selected species are understood more completely.

The ocean has been and will continue to be used for disposal of human wastes. Waste treatment and disposal must be carried out in ways that preserve the food, recreational potential, and resources of the seas. Many species are sensitive indicators of changes in water quality or eutrophication on local, regional, and even global scales. In addition, molecular probes and enzymatic tests may provide a rational basis to regulate the ocean disposal of industrial sludge, sewage, or radioactive substances. The expression of genes that respond to environmental stresses, such as changes in temperature, oxygen, and pollution (e.g., genes encoding heat shock proteins, cytochrome P450, metallothionein, and proteins), can potentially provide excellent sublethal indicators that could be used to monitor the impact of oceanic dump sites and other pollution problems.

As these sublethal indicators are refined for monitoring the impact of environmental pollution, methods can be automated to allow rapid and continuous analysis of water quality. This can be accomplished by the development of in situ sampling devices and reporting systems that either record the data directly or relay the information to a collecting station. The detecting system can be developed in the form of a biosensor or a microanalyzer system, and the information can be transferred via an acoustic, microwave, or optical system (National Research Council, 1993).

Food Safety

Food safety is of great importance to producers, processors, and consumers of seafood products. Improper disposal of human and industrial wastes and nonpoint source pollution have led to deteriorating coastal water quality, forcing the closing of shellfish and other fisheries, costing millions of dollars, and devastating local economies and businesses. Marine animals can harbor disease-causing organisms and contaminants (Ahmed, 1991). The present method of determining the safety of shellfish is based on antiquated techniques that require detection of coliform bacteria that often are not specific to human sewage. Techniques that rapidly and specifically detect contaminants and pathogens are available but have not been implemented. These capabilities need to be developed more fully and implemented for rapid and inexpensive monitoring of the food quality of marine resources.

Bioremediation

Contaminated waters, sediments, and living resources in areas from which seafood is harvested are an economic liability, a detriment to public health, and a social and recreational liability. Emerging technologies that include new instrumentation and biochemical, physiological, and molecular methods can be used to identify and exploit marine organisms to transform harmful contaminants to less harmful forms, improve water and sediment quality, and mitigate damage to coastal habitats. The Federal Coordinating Council for Science, Engineering, and Technology (FCCSET) budget document on biotechnology (FCCSET, 1992) identified bioremediation—the transformation of pollutants, toxic substances, and metals into less harmful forms by living organisms, primarily microorganisms—as a potential direction for marine biotechnology research. Essentially every country has urban waste treatment plants and industrial effluents that discharge billions of tons of polluted water into the lakes, rivers, estuaries, and coastal waters of the world. Aquatic microorganisms and plants have been used to treat human and agricultural wastes for hundreds of years. The use of natural microbes and aquatic plants continues to be an efficient and cost-effective method to treat urban waste. Molecular techniques may be useful for identifying and even producing species that are more effective. A number of studies suggest that mixed anaerobic bacterial communities are preferable to single species for the treatment of urban waste (Ahmed et al., 1984; Wetegrove, 1984).

Industrial effluents often contain man-made chemicals, toxins, and unique derivatives of naturally occurring compounds that may not be commonly degraded by aquatic microbes. In addition, massive spills of oil, toxins, and other

anthropogenic pollutants sometimes overwhelm the ocean's ability to cope efficiently with the huge excessive concentration of substrates, with disastrous consequences. Oil spills in Alaska and elsewhere are typical examples of the tremendous cost and ecological impact associated with our inability to cope with such spills. During the *Exxon Valdez* oil spill, nitrogen fertilization of small sections of selected intertidal areas resulted in the enhanced growth of a naturally occurring bacterium that could use the oil as a carbon source. Although this experiment was confined to small test areas, the results strongly suggest that supplying critical, rate-limiting nutrients to such bacteria may be one of the more efficient and cost-effective methods for dealing with the final cleanup phases of these disasters. In fact, over a decade ago, one of the first patented genetically engineered organisms was a multiplasmid bacterium for the rapid degradation of crude oil (Friello et al., 1976).

This approach is being applied to the degradation of other man-made toxins and pollutants. The U.S. Environmental Protection Agency has identified hundreds of chemical pollutants that are major problems in the aquatic environment because they are not readily degraded by naturally occurring microbes. The halogenated hydrocarbons, which make up the majority of these toxins, are particularly persistent in the aquatic environment and are toxic to a variety of organisms. A number of researchers have been using recombinant DNA techniques to create microorganisms that will degrade these toxins. Some success has been achieved in the degradation of halogenated hydrocarbons, phenols, and aromatic amines, using engineered microbes and mixed-species bioreactors containing a marine polysaccharide matrix (Portier and Fujisaki, 1986; Portier et al., 1987a; Sayler, 1990). For example, immobilization of microorganisms on a solid matrix has been shown to maximize their ability to degrade hazardous chemicals, such as chlorinated phenols and polychlorinated biphenyls. Properly engineered microbial bioreactors can remove such toxins continuously for periods of several months, with residual effluent levels of less than 100 parts per billion (see Attaway, 1989 and references therein).

MARINE ORGANISMS AS MODEL SYSTEMS

Scientific Questions

The ocean is a source of organisms that are used as model systems to address basic biology questions (Powers, 1989), some yet to be defined. Although automated identification of many marine taxa and assessment of functional diversity appears achievable (see Chapter 3), determination of the physiological status of marine organisms and the development of biological sensors is presently limited by the lack of fundamental information on the physiology, cell biology, and molecular biology of these organisms. In addition, the physiological basis for biological processes, such as recruitment, will require an understanding of the physiological and molecular basis for development, reproduction, nutrition, growth, speciation, and other fundamental information that is the foundation of the phenomenon that biological oceanographers wish to understand.

Marine organisms are used as models to address fundamental questions about the structure and function of cells, subcellular structures, organelles, organ systems, and whole organisms. A variety of molecular approaches can be used to elucidate modes of regulation and address questions about cell receptors, signal transduction, mechanisms of biosynthesis, and cellular transport. Some examples of representative areas that are already being actively analyzed at the molecular and biochemical levels are listed in Table 4-1 to illustrate the diverse, and potentially invaluable, biological systems for which marine organisms provide unique research advantages. Many more examples could be cited, and the list will become vastly expanded as our knowledge increases.

Major discoveries of fundamental biological processes—many important to human health and medical research—have come from studies of marine organisms. These include the discovery of cellular immunity, which was first recognized in starfish; discovery of the cellular and biochemical mechanisms controlling fertilization, which have been extensively studied in the sea urchin, abalone, and several other marine organisms; discovery of the mechanisms of nerve cell transmission for which giant cells of the squid have been particularly useful; discoveries of the mechanisms of nerve-to-nerve synaptic transmission, studied in the electric fish; insights into the cellular and molecular mechanisms responsible for learning and memory, for which certain marine snails present great experimental advantages; discoveries relating to neurosecretion and neuroendocrine regulation of reproduction, cardiac physiology, and daily rhythms of neuronal and physiological activities; and fundamental analysis of cell interaction, morphogenesis, and the molecular mechanisms of gene regulation in developing embryos. In this latter area sea urchin embryos have been a major experimental model for over a century, and ascidian, molluscan, and other embryos have been studied extensively as well.

Table 4-1. Examples of Biological Systems for Which Marine Animals Provide Unique Opportunities for Discovery.

| ORGANISM | BIOLOGICAL SYSTEM |
|--|--|
| Ctenophores | Biological-light producing system |
| Squid | Electrical impulse transmission system in nerves |
| Sea snails | Networks linking neuronal pathways, hormones, and behavior |
| Sea urchin embryos | Gene regulation systems |
| Clam, sea urchin, and tunicate eggs | Cell division and cytoskeletal control systems |
| Abalone and sea urchin gametes | Sperm-egg recognition systems |
| Sharks, hagfish, tunicates, and sea urchins | Novel and diverse immune systems that control bacteria and fungi |
| Abalone and sea urchin larvae | Cellular response and induction systems responding to environmental signals |
| Molluscan shell glands, echinoderm skeletogenic tissues | Cellular and molecular biofabrication systems for unique protein-mineral materials |

There is a need to understand the basic mechanisms by which genes control biological functions. The reproduction, development, differentiation, growth, recruitment, and life history patterns of all organisms are controlled by genes. The genes that encode the properties of cells and organisms are themselves controlled by the products of regulatory genes, some of which are sensitive to external environmental cues, others of which are activated by intercellular signals, while others operate according to predetermined genetic programs.

The complexities of gene regulation and the consequences for biological form and function have attracted the attention of molecular biologists for the past decade. Although major advances have been made in our understanding, much remains obscure on how genes determine biological functions, how they generate biological structures and materials, and how they control acclimation and adaptation. Among the most serious and general practical failures that will occur if our level of basic knowledge about molecular marine biology is not dramatically improved, are the following: (1) biotechnology research and development will miss opportunities (as noted below in Practical Implications); (2) we will be unable to predict the adaptive capability of marine species of economic and ecological importance to short-term changes in their environment or to global climate change; and (3) we will be unable to achieve a mechanistic understanding of the reproductive processes that control the population sizes of marine organisms or of the factors underlying the temporal and spatial variability of marine organisms. Without this kind of understanding, control of the ecological interactions of important marine species will never become possible.

Signal Molecules and Genes Controlling Reproduction, Development, and Growth

The reproduction, growth, and recruitment of marine organisms are controlled by molecular signals--molecular response mechanisms that can now be identified with the tools of molecular biology. It is possible, for the first time, to reliably control these processes in several valuable species, to attain improved efficiency and enhanced yields in mariculture, and to understand and predict variations in recruitment of valuable ocean stocks. The signals controlling these processes include molecules from the environment, molecules produced by other organisms, and molecules produced internally, that is, within the organism. *A major goal of molecular marine biology is the identification of these signal molecules and the genes that control their structures, their biosynthesis, their recognition, and the responses of these signals.* This is important because the signaling molecules and their receptors are among the most highly conserved proteins, and thus discovery of new signaling molecules and receptors in marine organisms will likely lead to the discovery of homologous molecules in vertebrates (e.g., Baxter and Morse, 1992). The point here is that the enormous diversity of

marine animal life offers a rich variety of opportunities for discovery. In addition, harnessing these signals, their receptors, their signal transducers, and the genes that encode them would have immediate practical applications in aquaculture, and advances in this area already have increased the possibilities of this industry in the United States. The near-term payoffs, however valuable, will constitute only a minor preview of the usefulness of this knowledge a few years from now, when much more sophisticated mariculture and biotechnological applications will become possible. This focus is reflected in the major goals of the new international programs on reproductive biology in aquaculture recently adopted by the International Union of Biological Scientists.

Neurobiology

Marine organisms play a central role in experimental research aimed at understanding cellular and molecular aspects of the central nervous system. Historically, the nerve impulse or action potential was discovered in a marine mollusc, the squid. Two examples serve to illustrate the importance of marine organisms in neuroscience research.

First, the marine snail *Aplysia* is widely used as a preparation for investigating the cellular regulation of behavior, including learning and memory. The neurons in *Aplysia* can be up to one millimeter in diameter, large enough to see with the naked eye. This special property allowed investigators to correlate the activity of individual nerve cells with specific behavioral patterns involved in locomotion, feeding, defensive movements, and reproduction. The neuronal circuitry--that is, how the cells are connected and how they communicate with each other--has been defined. When the animal experiences various stimuli the behavior is changed. The experience is encoded in the neuronal circuit and gives rise to a modified behavior. Since the circuitry is known, a cellular analysis of the behavioral modification has been possible to achieve experimentally. These studies have shown that the amount of chemical messenger released from a given cell and detected by a second cell is increased. This makes the connection between the cells stronger and is currently a fundamental cellular mechanism of memory (see review of Jung and Scheller, 1991).

A second group of marine organisms of importance to neuroscience research is the marine ray (primitive fish). These animals have been an electromotor system that is used as a defensive reflex or to shock prey prior to feeding. Nerve cells communicate by releasing chemicals called neurotransmitters from a specialized structure called a synapse. The density of synapses in the marine ray electric organ is about 100 times the concentration in human muscle and allowed the purification of a large variety of the molecules that comprise the synapse. These

molecules include the receptors for the neurotransmitter, the acetylcholine receptor (AChR). The AChR is currently the best-understood neurotransmitter receptor; it acts by opening a channel in the cell membrane so that ions can rapidly enter the cell when the signaling molecule (i.e., acetylcholine) binds to the receptor (reviewed by Karlin, 1991). This is a major mechanism of cellular response to external signals in the nervous system of all animals, including humans. Marine animals are valuable for neuroscience research aimed at understanding diseases as diverse as learning disabilities in children to Alzheimer's disease in the aged.

Differentiation and Pattern Formation

Morphological development is generated by regulated expression of genes early in the life cycle. It is these gene regulatory programs that must have arisen in evolution to account for the appearance of new life forms, by change or reassembly of preexisting regulatory programs that must have arisen in evolution to account for the appearance of new life forms, by change or reassembly of preexisting regulatory circuitry. These mechanisms are at the very heart of speciation in the ocean and reflect the evolutionary adaptation of higher taxonomic groups to changes in marine environmental parameters. That is, the biological and morphological properties of organisms are controlled by gene regulatory programs, and it is the different properties of organisms that enable them to adapt evolutionarily. Therefore, study of the molecular biology of gene regulation in differentiation intersects with the study of ecological adaptation and the evolutionary origin of organismic novelty. The union of these disciplines will require advanced molecular techniques now being developed that will enable identification, isolation, and analysis of key genes controlling cell type, pattern formation, and construction of adaptive traits, including in particular the isolation of regulatory gene products that control expression of these genes. The basic strategy has already been shown in the analysis of regulatory systems involved in the development of *Drosophila* and sea urchin embryos. Evidence obtained in these and other systems has demonstrated that evolution has functioned conservatively in utilizing the same active domains of genes and proteins in many different contexts. This has immediate and essential practical significance: basic discoveries of active regulatory molecules made in lower organisms, including invertebrates, often lead directly to the discovery of hitherto unknown homologous regulatory genes and proteins that are essential for normal vertebrate development and organ function.

Understanding development means understanding how multicellular organisms are progressively created through expression of the genetic programs carried in the egg and sperm nuclei (Davidson, 1990, 1991). Encoded in the genomic DNA is sequence information for all the proteins that endow the

differentiated cells of the organism with their special functional properties. The genome also contains the crucial regulatory information that ultimately ensures that the appropriate proteins are expressed in the appropriate spatial and temporal domains of the organism. Developmental gene regulation has been intensely studied in the sea urchin embryo, because of the practical advantages afforded by the availability of immense numbers of synchronously developing embryos; their permeability to radioisotopes; the ease of exogenous gene transfer into these eggs; accessibility to a variety of optical, cytological, biochemical, and molecular technologies; and because of their particularly simple, straightforward, early developmental process. Research on sea urchins demonstrates the type of approaches that could be extended to other organisms.

To achieve a mechanistic understanding of the developmental process is a fundamental goal of life sciences. Enough is known to perceive what will be required: isolation and characterization of marker genes that represent the various differentiated cell states of the developing organism; analysis of their gene regulatory systems; cloning and characterization of the regulatory proteins that determine their differential functions; and knowledge of the ligand-receptor interactions and signal transduction pathways by which the genetic and metabolic activities of each embryonic cell are related to those of adjacent cells. Knowledge of these problems is elemental to understanding the basic biological character of all metazoan organisms, plant and animal, as they are all the product of developmental processes.

Marine organisms have an essential role to play in the experimental endeavor to comprehend the mechanism of development. The ramifications of this endeavor extend all the way from basic enlightenment—how we get to be what we are—to high technology. First, marine organisms offer unique practical experimental advantages for the molecular study of development, as very briefly indicated above for what is currently the best-known marine example, the sea urchin. That is, by studying these organisms we can learn more, faster, and at immensely less cost, than we can by studying vertebrates, for instance. From one mouse, 50 to 80 eggs can be recovered, and these eggs can achieve only a very primitive state of development in vitro, that is, before implantation into the wall of the uterus. After this, it is technically very difficult (though of course not impossible) to have experimental access to them. One *Strongylocentrotus purpuratus* (the common California purple sea urchin) female will produce 20 million to 30 million eggs (of about the same dimensions as a mouse egg), which carry out complete development in vitro in the lab in about 72 hours. To isolate the rare molecules, present at only a few hundred to a few thousand molecules per nucleus, that regulate genes, it is immensely important to have available adequate quantities of developing material so that biochemical methods may be used. In addition, there are numerous negative attributes associated with exclusive reliance on mammalian

research models, ranging from their very high relative expense, to social attitudes. Second, marine metazoa offer by far the greatest biological diversity on the face of the earth. Diversity in developmental process underlies diversity in morphological form and hence of the diverse ecological adaptations that the morphologies of different embryos, larvae, and adults potentiate. This is an extremely important point: one of the most powerful approaches to discovering which functional aspects of molecular and cellular developmental processes are really fundamental, and which are peculiar to a given organism, is to make use of comparisons between organisms that develop differently. The marine environment offers many models for developmental molecular biology, and most of these have barely been superficially described. Their evolutionary relationships provide every imaginable degree of relatedness, from interspecific to interphyletic. The opportunities for startling new insights into developmental mechanisms that are awaiting exploitation in marine organisms cannot be overstressed.

A series of key model marine organisms should be chosen for comprehensive molecular-level study of developmental processes throughout the life cycle. These model systems should have several key features, including evidence of "high connectivity," that is, the relatedness of those molecular or cellular mechanisms that are uniquely accessible in the model system, to those basic mechanisms that are essential to progress in human medicine, or agriculture, mariculture, or ecological processes. In addition, requirements for the model systems should include some or all of the following, as appropriate: egg-to-egg laboratory culture must be feasible and not technically difficult; gene transfer into the egg should be possible; embryonic and other stages of material should be available in quantity; the material should be accessible for molecular biology, that is, it should be easy to prepare nucleic acids, nuclear extracts, cell types, mRNA; and clusters of evolutionarily related but morphologically distinct species should be available. At present, sea urchins and some teleost fish are the best known, but many additional invertebrate marine models need to be developed, including marine annelids, mollusks, other echinoderms, coelenterates, crustacea, and others. Marine organisms present ultimate versions of earlier evolutionary pathways, and a concerted effort to study the regulatory molecular biology of a set of diverse marine creatures will yield a depth of understanding of both evolutionary and developmental processes that far exceeds our present knowledge. This will undoubtedly affect, in turn, the state of knowledge in many of the most essential areas of bioscience and biotechnology.

To put the matter in the broadest light, we can regard the genomes of the organisms on this earth as the most valuable informational natural resource that exists. This reservoir of biological information has been growing for about 2 billion years. A very large part of this store of information is resident in marine creatures, about which our knowledge has so far lagged. The benefits that will derive from

deciphering and then applying this information are largely unforeseeable, just as not one could have predicted the fundamental transformations of society that followed from the discoveries of electricity or oil or calculus or the subatomic structure of the nucleus.

Techniques to Address the Scientific Questions

1. **Molecular Biological Technologies:** The key technologies are those required for core molecular biological investigations, as applied to marine organisms (described in Chapter 2). There are a series of special problems (and also special advantages) attendant on transfer of standard molecular biological methodologies to marine systems. Once the molecules are accessible, the techniques, methods, and instrumentation, are all in hand. These technologies are based fundamentally on recombinant DNA procedures for isolating, cloning, characterizing, and expressing genes and their products; on application of nucleic acid and protein physical chemistry; on the availability of a series of medium-level expensive instruments, including protein microsequencers, fluorimetry, spectrophotometers, DNA sequencers, PCR machines, and phosphorimaging devices; and on optical equipment capable of image processing, such as videomicroscope image processors equipped with fluorescent and differential interference contrast optics.
2. **Preparative Methods:** Development of marine molecular biology will, in the near future, require inventions of preparative methods, that is, knowledge of how to get from the organism or its cells to the molecules, genes, and proteins that must be studied. Among the preparations required are cell nuclei, nuclear extracts (that contain gene regulatory factors), mRNA, specific cell types, and cytoplasmic factors.
3. **Culture Methods:** A major area of technology for marine organisms that will be required is laboratory culture devices and procedures that will permit egg-to-egg culture of various marine species, including methods for raising embryos, larvae, juveniles, and adults in large quantities. This will also potentiate genetic techniques, including development of inbred strains and isolation and generation of useful and informative mutants; control of sexual differentiation and reproduction; and among the most important objectives, exploration of physiological responses to environmental factors of every kind, chemical, physical, and biological. Laboratory culture methods are also required for study of marine pathobiology and the immune systems of marine organisms.

Finally, there are several key technologies that need to be worked out for almost any sophisticated molecular biological application. These are development of methods for gene transfer and development of methods for tissue culture. Marine cell lines are yet very sparse, and even short-term culture methods are rare. However, for both biotechnological and basic research purposes this is an essential area, because cultured cells provide enormous opportunities for experimental manipulation, expression cloning, cell genetics, and tests of molecular function of all kinds. Gene transfer systems that are easy and sufficient exist so far only for some teleost fish and for sea urchin eggs. This technology is obviously required for analysis of gene regulatory systems, the key, basic area of research that will ultimately yield the ability to both understand and control gene expression.

Practical Implications

Human Health

Marine biologists, employing marine organisms as model systems, have not only changed our fundamental understanding of the basic biology of marine organisms but have also provided insight concerning the basic biology of humans, including the molecular mechanisms that regulate growth, reproduction, development, and the synthesis of a host of biomedically valuable metabolites. This research has resulted in many discoveries with significance for human health and medical research. Continued and future research in this field will almost certainly lead to opportunities in the development of new tools for diagnosing, treating, and preventing disease.

Marine Biotechnology

Numerous opportunities can be foreseen in the area of marine biotechnology. Marine biotechnology has scarcely begun to be exploited. Marine organisms present an enormous range of interesting and useful new materials, many of which are the products of a number of genes working together during the development of the animals, for example, protein-mineral complexes with fascinating tensile properties; medically active components, signal transduction devices, regulatory devices, optical devices, phosphorescent and luminescent systems, chemical and biochemical processes, prosthetic devices, underwater films, and fabrics. When scientists have a more complete understanding of the biology of marine organisms, the gene complexes and cell types that make these substances and biosystem elements will become accessible for controlled manufacture. These applications have the potential of effecting enormous transformations in the diversity and practical usefulness of commercial biotechnology.

CHEMICAL ECOLOGY

Scientific Questions

As in the terrestrial environment, the complex interactions of many marine plants and animals are mediated by chemical interactions. These chemical signals are important for maintaining stable community structures by regulating predator-prey interactions, establishing territories, and assisting in reproductive strategies. For example, predators find their prey, and potential prey avoid their predators, in part by detecting water-borne chemical cues; organisms avoid predation by generating distasteful or even toxic compounds; individuals find mates of their species by following trace odors through the sea; and swimming larvae of bottom-living animals are recruited to adult populations or find habitats where they can survive as adults by responding to specific chemical substances. In addition, many marine plants and animals have evolved solutions to unique problems in the sea, such as production of special skeletal materials and glues. For each of the phenomena cited here, interesting and unique questions arise: What is the chemical nature of the products involved? How are they produced and disseminated? How are they detected? Such compounds are potentially useful to humans.

In the ocean, predator-prey dynamics have been shown to be mediated by chemical effects such as attraction and deterrence (Rittschof and Bonaventura, 1986; Faulkner et al., 1990; Rittschof, 1990; Paul, 1992). Chemicals appear to be important, for example, in limiting food selection by zooplankters, placing in question the concept that measurements of chlorophyll alone adequately assess food availability and total productivity. Chlorophyll is an excellent measure of total primary producers; however, because all phytoplankton are not grazed upon equally, this measure may not be as useful as previously assumed until the relative food distribution of the species to zooplankton has been determined. Copepods

and bivalve mollusks select phytoplankton on the basis of its quality and often reject phytoplankters that produce toxins and other deterrent chemicals (Ward and Targett, 1989).

Despite their apparent inability to keep from releasing chemical clues to their whereabouts, many marine organisms use chemicals to avoid predation. For example, accumulating evidence indicates that the ink clouds of squids, octopuses, and sea hares probably do much more than confuse potential predators visually. In schooling squid they may chemically signal other members of the school of the need for flight (Gilly and Lucero, 1992), and in sea hares there are probably components that dull the senses of potential predators (Carefoot, 1987). Sea slugs, under attack by a predator, secrete specific compounds into their mucous trails that, when encountered by another slug, cause it to move rapidly away; such substances have been dubbed "alarm pheromones." Terrestrial examples of such compounds exist. Foul-tasting or toxic substances are known to be harmlessly ingested from plant sources by caterpillars of many butterfly species; their presence in the tissue of the caterpillars and butterflies serves as a deterrent to predatory birds. Similar events occur in the sea; sea hares stockpile toxic products from their algal foods and remain relatively predator-free as an apparent result (reviewed by Carefoot, 1987). On coral reefs, plants and animals that produce chemical deterrents and toxins are often less preyed upon. Soft-bodied invertebrates like sponges, ascidians, and soft corals produce large quantities of organic substances that provide elaborate chemical defenses (Paul, 1992). Recent studies demonstrate that even the minute larvae of a number of invertebrates are provided with chemical defenses from the mother's eggs.

Many sessile colonial invertebrates (sponges, ascidians, bryozoans, corals, soft corals) typically form colonies that enlarge by outward growth around the edges. As space is nearly always limiting in the habitats occupied by such organisms, they are in almost constant competition to gain new space and simultaneously keep from being overgrown by other colonies of their own or other species. Most encounters are carried out slowly by chemical means; in many such interactions, an unoccupied band occurs between interacting colonies where release of a chemical by one species leads to the retreat of another. In corals and ascidians it has been established that a colony can distinguish among other colonies those that were derived from the same sexually produced larva as itself and those that were not (reviewed by Rinkevich and Weissman, 1987; Grosberg, 1988). That is, they recognize "self" from "nonself."

Founding and maintaining populations of most bottom-living marine invertebrates depend on chemical cues released by one member of a species and detected by another. Many, if not most, mobile marine animals find their mates by following water-borne pheromones to their sources (e.g., Karplus, 1981; Hadfield

and Switzer-Dunlop, 1984; Zimmer-Faust et al., 1985). Spawning is often stimulated across populations by chemical means (Giese and Pearse, 1974-1987). Sperm find, recognize, and fuse with eggs as a function of chemical recognition (Miller, 1985). Free-swimming larvae use chemical cues from adults of their species to choose sites for settlement and metamorphosis (Crisp, 1974; Castro, 1978; Morse and Morse, 1984). It has been discovered for many marine invertebrates that continued recruitment of new settling larvae to an area depends on the presence of adults (Tegner and Dayton, 1977; Prince et al., 1988); recruitment potential is thus not just a function of larval availability, with important consequences for harvesting practices. Larvae of other species metamorphose in response to chemical cues from the habitat itself, including the specific plant or animal upon which it must feed after metamorphosis, or metabolites from microorganisms associated with the habitat (Morse and Morse, 1984; Hadfield, 1986; Hadfield and Pennington, 1990; Jensen and Morse, 1990; Pawlik, 1992).

Many marine organisms exist only in complex relationships with other organisms in various forms of symbiosis. Tropical corals and giant clams must harbor specific single-celled algae to exist; shipworms, actually a kind of clam, can feed on wood only when their digestive systems harbor specific protozoa containing specific cellulose-degrading bacterial symbionts; anemone fish live only among the tentacles of anemones; and parasites can survive only on or in specific host species. Many of these symbiotic relationships rely on the establishment of chemical cues in each new generation.

While there is evidence that specific chemicals are involved in the ecological interactions cited above, for only a small fraction are the chemicals themselves actually known, much less the mechanisms of their biological synthesis and detection. Chemical disruption of large areas of the intertidal or sea bottom by pollutants, oil spills, or even detergents used to clean up chemical spills can thus have grave and far-reaching consequences if they destroy, alter, or overwhelm the chemical signals upon which communities rely. Because very little is currently understood about such disturbances, an effort should be made to understand the impact of such disturbances on individuals, populations, species, and communities.

Unique conditions of aquatic realms have led to the development of unique solutions to such problems as maintenance of form and adhesion. Certain marine algae produce vast quantities of complex molecules with elastic properties to serve structural functions. Barnacles, mussels, and other marine organisms have evolved special glues that rapidly set in water. Interesting questions concern the molecular and cellular bases for the production of such chemical forms, although their usefulness in many applications has already been recognized (e.g., Rzepecki et al., 1991) (Table 5-1).

Table 5-1. Purification of Polyphenolic Protein from *Mytilus edulis* from Rzepecki et al. (1991).

| STEP | TOTAL PROTEIN, mg | TOTAL DOPA μmol | DOPA/ PROTEIN, $\mu\text{mol}/\text{mg}$ | YIELD, % | FOLD PURIFI- CATION |
|--------------------------|-------------------------|----------------------------------|--|----------|---------------------------|
| From 32 g of feet: | | | | | |
| S-1, perchloric acid | 210.0 | 71.3 | 0.34 | 100 | 1 |
| S-3, acetone precipitate | 66.7 | 41.6 | 0.62 | 58 | 1.8 |
| Sephadex G-200(a) | 29.4 | 30.5 | 1.04 | 43 | 3.1 |
| HPLC | 13.1 | 10.2 | 0.79 | 15 | 2.3(b) |

(a) Gel filtration was omitted for most mussel proteins.

(b) The specific activity of DOPA proteins decreases with time and manipulation (such as lyophilization) as a result of oxidation, but for *M. edulis* both gel filtration and HPLC chromatography were necessary for electrophoretic homogeneity. The deceptively low numbers for low purification result from the high relative proportion of polyphenolic protein in the initial extract.

Techniques to Address the Scientific Questions

The major questions in chemical ecology will yield answers more rapidly by the application of most of the methods of molecular biology described in Chapter 2. Specifically, isozyme techniques, as well as DNA sequence analysis, will be important in clarifying species identity questions, especially those involving specific interactions, be they predator-prey, symbiotic, or intraspecific. In some of the complex interactions involved in symbiosis, DNA sequence data will be necessary to determine which of a symbiotic pair is responsible for the production of compounds of interest. This can be accomplished by cloning and sequencing one or more of the genes involved in the synthesis of the compound or using other molecular probes when the symbiont can be cultured separate from its host. In situ hybridization methods will also greatly aid in such clarification, especially where microorganisms residing within larger multicellular organisms are suspected of being the source of compounds of interest.

Because many pheromones are peptides, cloning and sequencing will be important tools in locating the genes responsible for the synthesis of potentially useful pheromones. Peptides can be readily employed to generate specific

antibodies, and they, in turn, can be used to locate both a source organism and a specific site in the organism where the peptide is generated. This method will thus also be useful in determining whether a host or an endosymbiont is the source of a peptide. Eventually, recombinant DNA methods may be useful in splicing desired genes into microorganisms that can be readily mass generated.

Practical Implications

"Drugs from the Sea" and Agrichemicals

Over the past 30 years, a strong commitment has been made to utilize nature and its vast content of natural resources in the development of new drugs and agrichemicals. While terrestrial plants and microorganisms have been the traditional sources for well over half of today's medications, the potential of marine species is only now being recognized. Because most marine plants and animals are taxonomically and genetically distinct from those on land (Wesselds and Hopson, 1988), they often produce unique chemical compounds that provide the foundation for the next several decades of biomedical and agrichemical research. Recent studies of marine invertebrates have shown that they contain substances, many of which are defensive compounds, with significant potential for treatment of many human diseases. Marine invertebrate animals have already yielded substances that appear promising for the treatment of cancer (de Silva and Scheuer, 1980; Rinehart et al., 1981; Look et al., 1986).

Sponges and soft corals also produce biomedically important compounds, some of them showing significant antiinflammatory capabilities (Powers, 1990). Extracts of a Pacific sponge contain manoalide, a compound that reduces inflammation in both arthritis and asthma by specifically inhibiting the major enzyme involved in initiation of the inflammatory process. Pseudopterosins, compounds from a Caribbean soft coral, are also potent antiinflammatory agents.

Although it is expected that herbicidal and insecticidal activity will be discovered among the many marine defensive compounds, few studies have been initiated to explore the possibilities. In one case, however, the chemically defended soft coral (*Briareum polyanthes*) has been found to contain compounds that deter feeding by grasshoppers. Compounds used in chemical communication by marine organisms form a potentially rich source of repellents to insects and other invertebrate pests (e.g., nematodes and slugs) of commercially important plants.

The production of toxic compounds by marine organisms can have detrimental effects both on commercially important species and on humans. The

frequency of occurrence of "red tides" and other catastrophic toxic events in the sea appears to be increasing, causing growing concern in both environmental and public health contexts (Ahmed, 1991, and references therein). Human illness and death associated with toxic algal blooms and the consumption of contaminated shellfish include paralytic, neurotoxic, amnesic, and diarrhetic shellfish poisoning. These illnesses are caused by biotoxins produced by marine phytoplankton. In addition to humans, whales, dolphins, and seabirds that eat contaminated fish can become victims of the toxins, which are concentrated as they are passed up the food chain from phytoplankton to zooplankton to fish and ultimately to the final consumer. Consumption of contaminated fish can result in paralysis and even death. The causes and source organisms of toxic outbreaks are poorly known and almost totally unpredictable. Present monitoring techniques rely on measurements of toxins in the food product. It would be beneficial to predict future higher-level outbreaks by monitoring the abundance and distribution of the microorganisms responsible. Recent evidence reveals that many marine toxins are produced by microorganisms, including microalgae and bacteria, the distribution and population dynamics of which are almost totally unknown. Given the increasing frequency of poisoning from seafood and the growing dependence on seafood in the United States (Ahmed, 1991; Powers, 1990), it is imperative that we attain a full comprehension of the ecological and biochemical roles of marine microbial toxins and their sources. Recently, molecular methods have been developed to discriminate between toxic and nontoxic *Pseudonitzschia* species (Scholin et al., in press) that cause domoic acid accumulation. Such methods will allow reliable identification of microorganisms in environmental samples, thus potentially allowing problems to be recognized before they reach catastrophic levels.

Marine microorganisms also represent a vast and rich biomedical resource that remains virtually untapped (Fenical and Jensen, 1992). Minute free-living algae, bacteria, and fungi can be found in all parts of the ocean, and many more exist as symbionts on the surface and in the tissues of marine plants and animals. They represent a resource at least as diverse and prolific as the microorganisms from soil that form the basis of the production of most antibiotics. A few recent studies have already demonstrated the potential that exists: an unidentified deep-sea bacterium was shown to produce a new chemical class of compounds that inhibited tumor cell proliferation and replication of human immunodeficiency virus, the causative agent in AIDS (Gustafson et al., 1989). Several marine fungi have been studied and found to produce antibiotics and neuroactive substances. These few fundamental discoveries demonstrate the pharmacological resource potential of the microbial marine environment. Several impediments to progress in the area arise from our rudimentary understanding of marine microbial identities and ecology. In molecular biology lies the major hope for solving these problems and reaping the rewards to be gained from studying the biology and biochemistry of marine microorganisms.

Across the entire taxonomic spectrum, marine organisms produce compounds that are useful in a variety of human applications. They are sources of amino acids, vitamins, lipids, fats, waxes, sugars, polysaccharides, and pigments, as illustrated by the following examples.

Chitin

Chitin, a complex nitrogenous polysaccharide, is one of the most abundant biopolymers in the world, and in the sea it makes up a large portion of the exoskeleton of many marine invertebrates, especially crustaceans. Chitin has an untapped commercial potential because it can be used for a large variety of industrial applications, including adhesives, chelating agents, paper and textile additives, and structural matrices, and its potential for promoting wound healing and other biomedical applications is enormous (Muzzarelli et al., 1986).

Complex Polysaccharides

Complex polysaccharides from marine algae, especially agar, carrageenans, and alginates, already form the basis of a \$300 million per year industry for their uses as emulsifiers, stabilizers, and thickeners and for their biomedical applications. As the demand for these products grows and the natural supply declines, the necessity for genetically engineered, high-quality, fast-growing algae will become apparent, and the current U.S. dependence on foreign sources will become less tolerable. Already the most up-to-date methods of molecular and cellular biology are being utilized to produce new, culturable strains of high-quality, agar-producing algae. There are also examples of ecologically and physiologically important plant pigments (phycobilins, carotenoids) of current and future commercial use in the pharmaceutical and food industries.

Vitamins and Other Essential Nutrients

A number of commonly cultured marine algae are excellent sources of a broad spectrum of vitamins and other essential nutrients, contributing to an industry whose sales now exceed \$2 billion per year for human and animal uses. Vitamin B₁₂ and pantothenic acid are commonly excreted by several species of blue-green algae, opening the possibility for continuous culture methods for producing these vitamins. Algae are already a commercial source of food additives, including β -carotene, glycerol, amino acids, alcohols, and other hydrocarbons (Chapter 3; Ben-Amotz and Arvon, 1990; Behrens and Delente, 1991; Bubrick, 1991). However, only about 60 of the estimated 22,000 to

26,000 living algal species have been surveyed for their content of vitamins or other useful chemicals or bioactive metabolites. They clearly provide an untapped resource of biological diversity that merits extensive research.

Adhesives

Because they evolved in and still inhabit hostile environments where wave action requires a truly tight grip, many marine mussels, barnacles, and other invertebrates have incredibly strong glues that serve this purpose. In addition, these glues are secreted and "set" in a watery medium, a property lacking in most commercially available adhesives. One of these glues has been isolated and structurally characterized; it is a protein with unique structural modifications that allow it to harden underwater. Attempts are now being made to produce the protein with recombinant methods. This type of glue will be in high demand for a large spectrum of aqueous cement applications, including repair of broken bones and dental repairs.

Microbial Products and Biochemical Processes

The existence of bacteria living in very high temperature water at deep-sea hydrothermal vents opens the possibility of manipulating them for commercial purposes, especially isolating—and probably genetically engineering—their heat-resistant enzymes. The ability of these enzymes to withstand very high temperatures could greatly enhance chemical reaction rates in commercial applications, and promising results have already been obtained. In fact, a new form of the enzyme *tac* polymerase that makes it possible to do the PCR at high temperatures (discussed in Chapter 2) was cloned from a thermophilic bacterium isolated from a hydrothermal vent and is now commercially produced by Stratagene (La Jolla, Calif.). A number of hyperthermophilic bacteria have been isolated and cultured at temperatures between 90° and 110°C, and enzymes of several types have been isolated from them and found to be active and stable at elevated temperatures (Brown et al., 1990). Some of these enzymes have been patented for future commercial applications. The fact that these enzymes are stable at high temperatures makes them particularly useful for chemical processes that require reactions at or near 100°C.

Some of the bacteria of hydrothermal vent communities have another potentially very useful attribute: the ability to oxidize hydrogen sulfide and use the energy to fix carbon dioxide into complex organic compounds. Virtually all other ecosystems on earth derive such energy from the sun via photosynthesis. Hydrothermal vent bacteria should be useful for converting industrial hydrogen

sulfide into less toxic products and for desulfurizing coal. Other bacteria in these hydrothermal vent communities reproduce extremely rapidly and produce methane (Brock, 1985), offering the potential for more efficient biomass conversion into methane than is obtained with low-temperature bacteria. Clearly, tremendous possibilities exist for the use of hydrothermal vent microorganisms in the chemical, energy, biomedical, and pollution treatment industries.

This report highlights many important sources and types of chemical/biochemical substances to be found in studies of "chemical ecology." Textbooks, reviews, and classical research papers abound with examples of chemical interactions in marine organisms that provide promise as opportunities for economic utilization (e.g., Attaway, 1988, 1989; Ahmed and Attaway, 1987; Ahmed, 1991; Attaway and Zaborsky, 1992; Colwell, 1983; Colwell and Zilinskas, 1993; Powers 1990; Zaborsky, 1993).

RECOMMENDATIONS

This report defines critical scientific questions in marine biology and biological oceanography, describes the molecular technologies that could be used to answer these questions, and discusses some of the potential implications and economic opportunities that could improve the international competitive position of the United States in the rapidly growing area of marine biotechnology. The committee recommends that the federal government commit to providing the infrastructure necessary to use the techniques of molecular biology in the marine sciences. In particular, the committee makes recommendations in four areas.

Research Needs

This report identifies a suite of critical scientific questions in the fields of marine biology and biological oceanography. From these important questions, the committee has identified seven basic research topics that it believes could immediately benefit from increased attention and more appropriate facilities for carrying out molecular approaches.

- **Quantification of inter- and intraspecific genetic variations for assessing species biodiversity, population structure, migratory movements, and gene flow. Data resulting from such assessments should be archived in a readily accessible format. Of particular importance are DNA-based data for commercially important fishes, other species that are indicators of ecosystem health, and critically endangered species.**
- **Clarification of the role of marine viruses in marine ecosystems in light of their potential importance in ocean processes.**

- **Determination of effects of the environment on physiology and adaptation, especially the mechanisms regulating gene expression at the molecular level.**
- **Elucidation of metabolic pathways in marine organisms that lead to the synthesis and degradation of secondary metabolites and contaminants.**
- **Investigation of the role of chemical signals in the marine environment, including their chemical nature, detection, and potential usefulness to humans.**
- **Investigation into the basic biology of a series of "keystone" marine organisms in order to develop techniques for assessing their physiological status in relation to recruitment processes, biogeochemical cycles, and other ocean processes.**
- **Investigation of how eutrophication, toxic discharges, global change, and other human-induced environmental disruptions affect the abundance, distribution, and ecological success of species (i.e., biological diversity).**

This effort would include development of specific molecular probes for (1) proteins important in physiology, particularly to study environmental effects on photosynthesis, respiration, growth, and reproduction of phytoplankton; (2) bacteria and macrophytes important for understanding cycling of biologically important elements (carbon, nitrogen, phosphorus, and sulfur) and control of global cycles; (3) phytoplankton and bacteria that produce toxic blooms; and (4) commercially and ecologically important fish species. In addition, as new molecular data become available, numerical models for ecological, physiological, climatic, and other processes will need to be modified to accommodate this new data.

Technology Development, Technology Transfer, and Infrastructure

Some advancement of fundamental biological knowledge has come through the development of new molecular technologies. The development of these technologies has occurred outside the marine sciences. Technologies to solve many of the complex problems faced by marine scientists, therefore, either do not exist or must be redesigned for oceanography. Thus, more effective mechanisms for encouraging rapid transfer of molecular biological technologies into marine science laboratories must be developed, if this field is to fulfill its potential. There are three aspects of this process: development of new technologies, transfer of new technologies into the marine sciences, and provision of infrastructure (e.g., facilities, equipment, study organisms).

Technology Development: The technology development upon which marine science depends will occur in many places—commercial, government, and academic laboratories—with a wide range of foci, including biomedical research, agricultural research, and marine science research. Although the committee does not make recommendations regarding the mechanisms required to accomplish this development, it does make a number of recommendations identifying research areas that would benefit from technology development.

- **Couple molecular methods with new detection systems and computer-controlled robotic systems, so that large numbers of samples can be analyzed effectively.**
- **Determine the potential usefulness of marine viruses as vectors for the genetic manipulation of marine organisms.**
- **Choose a series of key model marine organisms for comprehensive molecular-level study of developmental processes throughout their life cycles.**

Technology Transfer: Technology transfer implies the application to marine science of techniques developed originally for use in other areas. Components of technology transfer include education and training, as well as mechanisms to adapt molecular techniques developed outside marine science for the study of marine organisms and processes. The technologies and approaches of molecular biology could contribute to existing federally funded initiatives, such as the National Science Foundation's Joint Global Ocean Flux Study, the Ridge Interdisciplinary Global Experiment, the Land Margin Ecosystems Research program, and the Global Ocean Ecosystems Dynamics program. Specific recommendations call for:

- **Developing technology to enable manipulation of organisms on board ships and in the laboratory under in situ environmental conditions.**
- **Maintaining and strengthening research fellowships and traineeships in molecular marine biology. A training program for midcareer biological oceanographers and marine biologists who desire to use molecular biological techniques in their research should be established. The National Oceanic and Atmospheric Administration would benefit greatly by establishing a marine biotechnology graduate fellowship program through its Sea Grant Program, with an applied science focus to study environmental change, land-sea interactions, water quality and productivity, habitat quality and restoration, and health of living resources.**

Infrastructure: Appropriate infrastructure will be necessary to promote development of new technologies and to provide opportunities for their use in marine science. Infrastructure modernization will be necessary for many marine research and teaching facilities in the United States. It is recognized that responsibility for these tasks belongs in some cases to the federal government and in others to universities, scientific societies, industry, and individual scientists. There is an opportunity for collaborative efforts among government, academic institutions, and industry in molecular marine biology research and infrastructure development. Other disciplines of marine science have found that regional or national facilities provide a means for efficient sharing of expensive resources. Examples include the University-National Oceanographic Laboratory System fleet, the *JOIDES Resolution* drillship, and the Woods Hole Accelerator Mass Spectrometer facility. National or regional facilities could provide needed upgrading of the infrastructure underlying U.S. marine biological research. The committee makes three specific infrastructure-related recommendations:

- **Increase the availability of equipment and instrumentation needed to enable marine ecologists and biological oceanographers to perform molecular studies.**
- **Improve the basic infrastructure of undergraduate and graduate teaching laboratories to include modern state-of-the-art instrumentation, and provide new facilities and laboratories where necessary.**
- **Modernize U.S. facilities for the culture of marine organisms to ensure a supply of critical microorganisms, marine algae, plankton, and marine animals for the studies recommended above.**

Various agencies, particularly the National Institutes of Health and the National Science Foundation, have worked to establish culture facilities. Relatively few marine organisms can now be cultured through their life cycles, and most facilities still rely on capture and maintenance of wild organisms. With wild-caught animals, the genetic background, reproductive state, disease condition, and nutritional state are largely unknown, which presents numerous disadvantages. Modernized facilities should include capabilities for mariculture, isolation, and cultivation of microorganisms; specialized laboratories for animal health; equipment for molecular-biology-based research; modern microscopy; automated sampling; and computer access to data.

Public and Commercial Applications

A mechanism is needed to promote collaborative partnerships among federal agencies, academic marine scientists, and private industry and to permit appropriate research findings on marine organisms to be rapidly transferred into the private sector for commercialization. Private sector participation in funding research efforts and infrastructure could speed the development of bioremediation and environmental monitoring methodologies, as well as promote basic research on the biochemistry of novel compounds and metabolites that might be useful for biomedical applications. The private sector could also benefit from participation in a partnership with marine scientists and federal agencies to help support studies of marine biodiversity, which has the potential for exciting discoveries of biomedically important and/or environmentally useful organisms and compounds. In particular, the committee has identified three areas where the application of molecular biological techniques may lead to improvements in public health and/or the development of new products:

- **Better methods for screening contaminated waters, sediments, and seafood should be developed. In addition, techniques are needed to detect indicators of chemical and biological contamination in order to monitor the safety of the marine environment and its living resources.**
- **Bioremediation methods should be explored using marine organisms or their gene products.**
- **Rapid screening methods for identifying and isolating biomedically useful compounds from marine organisms should be developed.**

Coordination of Support

The National Science and Technology Council, which replaces the Federal Coordinating Council for Science, Engineering, and Technology (FCCSET), provides a mechanism to integrate the biotechnology funding of federal agencies. FCCSET had been developing a coordinated national effort in biotechnology and has included marine aspects, explaining the opportunities that will accrue by fostering research in marine biotechnology and by promoting interagency cooperation. The 1992 FCCSET report states, "It is clear that Federal efforts in research laying the basis for further development of marine biotechnology must be intensified, to take advantage of largely untapped resources and to prepare skilled technicians and scientists for international competition in developing bio-industries in the 21st century. Just as important, application of biotechnological techniques is essential to elucidating oceanic processes affecting or controlling global processes."

According to the same report (p. 58), the entire federal investment for fiscal year 1992 in marine biotechnology was about \$44 million. Although the U.S. investment in marine biotechnology is significant, it is modest compared with the efforts of some of America's most competitive international trading partners. Sensing the tremendous economic opportunity for marine biotechnology in the future, Australia, Norway, France, Germany, Israel, Japan, China, Taiwan, Thailand, and other European and Asian countries are spending hundreds of millions of dollars on marine biotechnology research and development (Myers and Anderson, 1992; Yuan and Hsu, 1993; Zaborsky, 1993). As pointed out in the FCCSET report, Japan's investment alone is currently \$180 million annually. Although exact figures are not available for each of the other countries, it is clear that their effort is very substantial (Yuan and Hsu, 1993; Zaborsky, 1993).

The members of the Committee on Molecular Marine Biology feel strongly that, in order to answer the scientific questions posed in this report, achieve the scientific potential afforded by the techniques of molecular biology, and enhance the development and international competitiveness of the United States in the area of marine biotechnology, several actions will be necessary:

- **Federal agencies, private industry, and academic scientists should work more closely together.**
- **The federal government should make an immediate long-term commitment to support molecular marine biology and biotechnology research and development.**
- **Adequate facilities and committed researchers are essential for scientific advances; the federal government, private sector, and scientists should work together to ensure that these physical and human resources will be available.**
- **Oceanographic programs with biological components and individual scientists working on questions amenable to molecular approaches should be targeted for encouragement and support.**

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